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<p>(54) Title: HYDROXAMIC ACIDS SUBSTITUTED BY HETEROCYCLES USEFUL FOR INHIBITION OF TUMOR NECROSIS FACTOR</p> <p style="text-align: center;"> (I) </p> <p>(57) Abstract</p> <p>Compounds of formula (I), wherein: n is 1 to 6; Het is a nitrogen containing ring fused to the benzene ring on two adjacent carbon atoms to form a bicyclic ring system which ring system may be optionally substituted; R¹ is hydrogen, C₁-alkyl, C₂-alkenyl, C₂-alkynyl, C₃-cycloalkyl, aryl, heteroaryl, heterocyclyl, arylC₁-alkyl, heteroarylC₁-alkyl or C₃-cycloalkylC₁-alkyl; R² is C₁-alkyl, C₂-alkenyl, arylC₁-alkyl, heteroarylC₁-alkyl or the side-chain of a naturally occurring amino acid; R³ is hydrogen, C₁-alkyl, C₃-cycloalkyl, C₄-cycloalkenyl, arylC₁-alkyl, heteroarylC₁-alkyl or heterocyclylC₁-alkyl; R⁴ is hydrogen or C₁-alkyl; or R³ and R⁴ together with the nitrogen atom to which they are joined form a heterocyclic ring; wherein any group or ring, in R¹-R⁴, is optionally substituted; and pharmaceutically acceptable salts and <i>in vivo</i> hydrolysable esters thereof, are described as inhibitors of the production of Tumour Necrosis Factor and/or one or more matrix metalloproteinase enzymes. Compositions containing them and their preparation are also described.</p>			

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HYDROXAMIC ACIDS SUBSTITUTED BY HETEROCYCLES USEFUL FOR INHIBITION OF TUMOR NECROSIS FACTOR

This invention relates to hydroxamic acid compounds and in particular to such compounds with a heterocyclicalkoxy substituent. This invention further relates to processes 5 for preparing such compounds, to pharmaceutical compositions containing them and to their use in methods of therapeutic treatment.

The compounds of this invention are inhibitors of the production of TNF (Tumour Necrosis Factor) which is believed to be formed by the cleavage of a pro-form, or larger precursor, by the enzyme pro-TNF Convertase. Applicants believe that the compounds of the 10 present invention inhibit TNF production by mechanisms which include inhibition of pro-TNF Convertase. The term 'TNF' is used herein to refer to Tumour Necrosis Factor in general but, in particular, to TNF α .

The compounds of this invention will be useful in the treatment of disease or medical conditions in which excessive TNF production is known to give rise via a cascade of 15 processes to a variety of physiological sequelae including the production of physiologically-active eicosanoids such as the prostaglandins and leukotrienes, the stimulation of the release of proteolytic enzymes such as collagenase, the activation of osteoclast activity leading to the resorption of calcium, the stimulation of the release of proteoglycans from, for example, cartilage, the stimulation of cell proliferations and to angiogenesis. It is also known that, in 20 certain cellular systems, TNF production precedes and mediates the production of other cytokines such as interleukin-1 (IL-1) and interleukin-2 (IL-2) which are also believed to contribute to the pathology of disease states such as inflammatory and allergic diseases and cytokine-induced toxicity. Excessive TNF production has also been implicated in mediating or exacerbating the development of various inflammatory and allergic diseases such as 25 inflammation of the joints (especially rheumatoid arthritis, osteoarthritis and gout), inflammation of the gastrointestinal tract (especially inflammatory bowel disease, ulcerative colitis and gastritis), skin disease (especially psoriasis, eczema and dermatitis) and respiratory disease (especially asthma, bronchitis and allergic rhinitis), and in the production and development of various cardiovascular disorders such as myocardial infarction, angina and 30 peripheral vascular disease. Excessive TNF production has also been implicated in mediating complications of bacterial, fungal and/or viral infections such as endotoxic shock, septic

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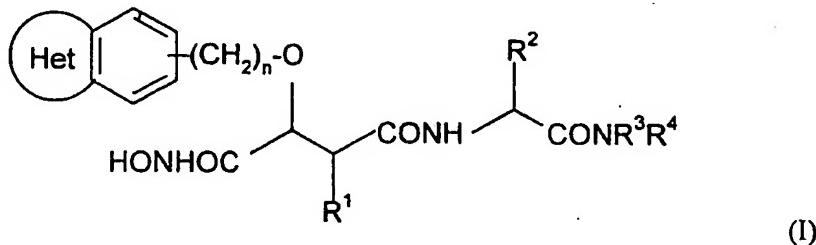
shock and toxic shock syndrome. Excessive TNF production has also been implicated in mediating or exacerbating the development of adult respiratory distress syndrome, diseases involving cartilage or muscle resorption, Paget's disease and osteoporosis, pulmonary fibrosis, cirrhosis, renal fibrosis, the cachexia found in certain chronic diseases such as 5 malignant disease and acquired immune deficiency syndrome (AIDS), tumour invasiveness and tumour metastasis and multiple sclerosis.

The compounds of the invention may also be inhibitors of one or more matrix metalloproteinases such as collagenases, stromelysins and gelatinases. Thus they may also be of use in the therapeutic treatment of disease conditions mediated by such enzymes for 10 example arthritis (rheumatoid and osteoarthritis), osteoporosis and tumour metastasis.

The present invention provides novel compounds which have activity as inhibitors of TNF and/or are inhibitors of one or more matrix metalloproteinase enzymes.

Accordingly the present invention provides a compound of the formula (I):

15



wherein:

n is 1 to 6;

20 Het is a nitrogen containing ring fused to the benzene ring on two adjacent carbon atoms to form a bicyclic ring system which ring system may be optionally substituted;

R¹ is hydrogen, C₁₋₈alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl, heterocyclylC₁₋₆alkyl or C₃₋₈cycloalkylC₁₋₆alkyl;

25 R² is C₁₋₆alkyl, C₂₋₆alkenyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl or the side-chain of a naturally occurring amino acid;

R³ is hydrogen, C₁₋₆alkyl, C₃₋₈cycloalkyl, C₄₋₈cycloalkenyl, arylC₁₋₆alkyl,

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- R⁴ is heteroarylC₁₋₆alkyl or heterocyclC₁₋₆alkyl;
 R⁴ is hydrogen or C₁₋₆alkyl; or R³ or R⁴ together with the nitrogen atom to which they
 are joined form a heterocyclic ring;

- 5 wherein any group or ring, in R¹-R⁴, is optionally substituted;
 or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof.

"Aryl" in the terms "aryl" and "arylC₁₋₆alkyl" typically means phenyl or naphthyl, preferably phenyl. "Heteroaryl" in the terms "heteroaryl" and "heteroarylC₁₋₆alkyl" means an aromatic mono- or bicyclic 5-10 membered ring with up to five ring heteroatoms selected

- 10 from nitrogen, oxygen and sulphur. Examples of 'heteroaryl' include thiienyl, pyrrolyl, furanyl, imidazolyl, thiazolyl, pyrimidinyl, pyridinyl, indolyl, benzimidazolyl, benzthiazolyl, quinolinyl and isoquinolinyl. "Heterocycl" in the terms "heterocycl" and heterocycl-C₁₋₆alkyl" means a non-aromatic mono- or bicyclic 5-10 membered ring with up to five ring hetero atoms selected from nitrogen, oxygen and sulphur. Examples of 'heterocycl' include
 15 pyrrolidinyl, morpholinyl, piperidinyl, dihydropyridinyl and dihydropyrimidinyl.

Any group or ring in R¹-R⁴ may be optionally substituted, for example by up to three substituents which may be the same or different. Typical substituents include: hydroxy, C₁₋₆alkoxy for example methoxy, mercapto, C₁₋₆alkylthio for example methylthio, amino, C₁₋₆alkylamino for example methylamino, di-(C₁₋₆alkyl)amino for example dimethylamino,
 20 carboxy, carbamoyl, C₁₋₆alkylcarbamoyl for example methylcarbamoyl, di-C₁₋₆alkylcarbamoyl for example dimethylcarbamoyl, C₁₋₆alkylsulphonyl for example methylsulphonyl, arylsulphonyl for example phenylsulphonyl, C₁₋₆alkylaminosulphonyl for example methylaminosulphonyl, di-(C₁₋₆alkyl)aminosulphonyl for example dimethylamino-sulphonyl, nitro, cyano, cyanoC₁₋₆alkyl for example cyanomethyl, hydroxyC₁₋₆alkyl for example
 25 hydroxymethyl, aminoC₁₋₆alkyl for example aminoethyl, C₁₋₆alkanoylamino for example acetamido, C₁₋₆alkoxycarbonylamino for example methoxycarbonylamino, C₁₋₆alkanoyl for example acetyl, C₁₋₆alkanoyloxy for example acetoxy, C₁₋₆alkyl for example methyl, ethyl, isopropyl or tert-butyl, halo for example fluoro, chloro or bromo, trifluoromethyl, aryl for example phenyl, arylC₁₋₆alkyl for example benzyl, aryloxy for
 30 example phenoxy, arylC₁₋₆alkoxy for example benzyloxy, heteroaryl, heteroarylC₁₋₆alkyl, heterocycl and heterocyclC₁₋₆alkyl. The term "side chain of a naturally occurring amino

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acid" means the side chain X of an amino acid $\text{NH}_2\text{-CHX-COOH}$. Suitable amino acids include alanine, arginine, aspartic acid, cysteine, asparagine, glutamine, histidine, homoserine, isoleucine, leucine, lysine, methionine, norleucine, norvaline, ornithine, serine, threonine, tryptophan, tyrosine and valine.

5 The compounds of the present invention possess a number of chiral centres, at the carbon atom adjacent to the HONHOC- group, at $-\text{CHR}^2-$, at $-\text{CHR}^1-$ (when R^1 is not hydrogen) and possibly in the variables $\text{R}^1\text{-R}^4$. The present invention covers all diastereoisomers and mixtures thereof that inhibit TNF Convertase and/or inhibit matrix metalloproteinase enzymes.

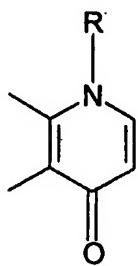
10 n is 1 to 6, preferably n is 1 or 2 forming a methylene or ethylene moiety. Most preferably n is 1 forming a methylene moiety.

Suitably Het is a ring containing one or two ring nitrogen atoms. Suitably Het, together with the two fused carbon atoms, is a 5- or 6-membered ring. Therefore in a particular aspect Het is a 5- or 6-membered ring containing one or two ring nitrogen atoms.

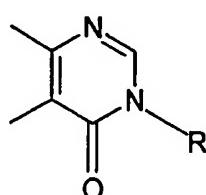
15 In one aspect Het and the benzene ring to which it is fused form a bicyclic heteroaryl ring system for example quinoline, quinazoline, phthalazine, cinnoline, isoquinoline, indole, isoindole or indazole. In a further aspect Het and the benzene ring to which it is fused form quinoxaline.

Preferably benzene-Het is quinoline, quinazoline or isoquinoline.

20 In another aspect Het is a pyridone or pyrimidone ring such as of the sub-formulae (i)-(iii):



(i)



(ii)



(iii)

- 5 -

wherein R is hydrogen or C₁₋₆alkyl [Compounds wherein R is hydrogen may be regarded as keto-tautomers of the corresponding aromatic system substituted by hydroxy].

Preferably Het is a pyridone or pyrimidone ring of the sub-formula (ii) or (iii).

5 In another further aspect Het is a tetrahydropyridone or tetrahydropyrimidone ring forming for example a bicyclic tetrahydroquinolone system. Het may also be an oxazine forming a benzoxazine ring system.

In yet a further aspect Het is a five-membered ring for example an oxazole, thiazole, pyrrole or dihydropyrrole forming for example a benzoxazole, benzthiazole, indole or
10 dihydroindole ring system.

The bicyclic ring system formed by Het and the benzene ring may be optionally substituted. on either ring, by up to three substituents which may be the same or different. Typical substituents include those described hereinbefore in relation to any group or ring in R¹-R⁴. In particular, preferred substituents for the benzene-Het fused bicyclic ring system are
15 C₁₋₆alkyl, halo, hydroxy, amino, C₁₋₆alkylamino and di-C₁₋₆alkylamino.

The -O(CH₂)_n- moiety may be linked to any convenient carbon atom of the benzene ring.

Particularly preferred values for the bicyclic ring system are quinoline, isoquinoline, quinazoline, 1-methyl-2-oxo-1,2-dihydroquinoline, 2-methyl-4-hydroxyquinazoline and
20 2-methyl-4-hydroxy-7-bromoquinazoline. Further preferred values for the bicyclic ring system are benzoxazole and 2-methylbenzothiazole.

Particular groups for R¹ include C₁₋₈alkyl for example isopropyl, n-propyl, isobutyl, sec-butyl, n-butyl, tert-butyl, isopentyl, n-pentyl, hexyl, heptyl or octyl; C₁₋₈alkyl interrupted by an oxygen or sulphur atom for example methoxypropyl, ethoxyethyl, propoxymethyl,
25 ethylthioethyl or methylthiopropyl; phenylC₁₋₆alkyl for example benzyl, phenethyl, phenylpropyl or phenylbutyl; arylC₁₋₆alkyl interrupted by oxygen or sulphur for example benzyloxybutyl or benzyloxypropyl; C₃₋₈cycloalkyl for example cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl; or C₃₋₈cycloalkylC₁₋₆alkyl for example cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyl.

30 Preferably R¹ is isobutyl.

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There is a chiral centre at -CHR¹- (when R¹ is not hydrogen); it is preferred that this centre has the configuration indicated in formula (II) hereinafter. For most values of R¹ this centre will have the R-stereochemistry under the Cahn-Prelog-Ingold sequence rules.

Particular groups for R² include C₁₋₆alkyl for example methyl, ethyl, isopropyl, n-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, isopentyl, n-pentyl or hexyl; C₁₋₆alkyl interrupted by an oxygen or sulphur atom for example methoxyethyl, methoxypropyl, methylthioethyl or 1,1-dimethylmethylthiomethyl (MeSCMe₂-); or phenylC₁₋₆alkyl for example benzyl or phenethyl.

Preferably R² is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl with tert-butyl being most preferred.

The chiral centre at -CHR²- preferably has the configuration indicated in formula (II) hereinafter. For most of R² this centre will have the S-stereochemistry.

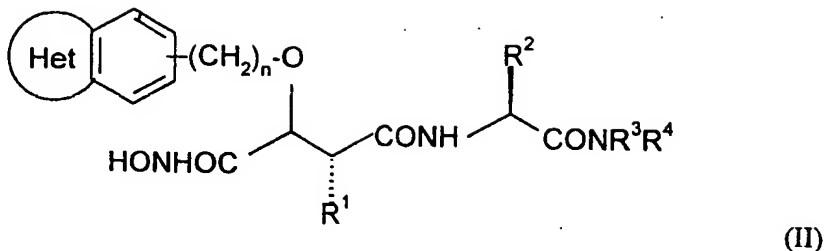
Particular groups for R³ include C₁₋₆alkyl for example methyl, ethyl, n-propyl, isopropyl, tert-butyl or n-butyl; C₁₋₆alkyl interrupted by an oxygen or sulphur atom for example hydroxyethyl, methoxyethyl, methylthioethyl or ethoxyethyl; C₂₋₆alkyl substituted by either amino, C₁₋₆alkylamino or di-C₁₋₆alkylamino; phenylC₁₋₆alkyl for example benzyl, phenethyl or phenylpropyl; heterocyclicalkyl for example 2-morpholinoethyl, 2-piperazinoethyl, 2-(N-methylpiperazino)ethyl or 2-piperidinoethyl; or C₃₋₈cycloalkylC₁₋₆alkyl for example cyclopropylmethyl, cyclobutylmethyl or cyclopentylmethyl.

Preferably R³ is methyl, ethyl, n-propyl, isobutyl, tert-butyl or benzyl. Of these methyl is most preferred.

Particular groups for R⁴ are hydrogen and C₁₋₆alkyl for example methyl or ethyl. Preferably R⁴ is hydrogen.

A particularly suitable class of compounds of the present invention is that of formula (II):

- 7 -



wherein n, Het, R¹, R², R³ and R⁴ are as hereinbefore defined.

- A preferred class of compounds of the formula (II) is that wherein n is 1; Het and the
- 5 benzene ring to which it is fused is quinoline, quinazoline, or isoquinoline, any of which is unsubstituted or substituted by one or two groups selected from halogen for example chloro, bromo or fluoro, C₁₋₆alkyl for example methyl, isopropyl or tert-butyl, C₁₋₆alkoxy for example methoxy, hydroxy, amino, C₁₋₆alkylamino for example methylamino or di-C₁₋₆alkylamino for example dimethylamino; R¹ is isobutyl; R² is isobutyl, tert-butyl or benzyl; R³ is methyl,
 - 10 ethyl, n-propyl, isobutyl, tert-butyl, 2-dimethylaminoethyl or benzyl; and R⁴ is hydrogen or methyl.

- A further preferred class of compounds of the formula (II) is that wherein n is 1; Het is of the sub-formula (ii) or (iii) wherein either of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro, bromo or fluoro, C₁₋₆alkyl for
- 15 example methyl, isopropyl or tert-butyl, C₁₋₆alkoxy for example methoxy, hydroxy, amino, C₁₋₆alkylamino for example methylamino or di-C₁₋₆alkylamino for example dimethylamino; R¹ is isobutyl; R² is isobutyl, tert-butyl or benzyl; R³ is methyl, ethyl, n-propyl, isobutyl, tert-butyl, 2-dimethylaminoethyl or benzyl; and R⁴ is hydrogen or methyl.

- Suitable pharmaceutically acceptable salts include acid addition salts such as
- 20 hydrochloride, hydrobromide, citrate and maleate salts and salts formed with phosphoric and sulphuric acid. In another aspect suitable salts are base salts such as an alkali metal salt for example sodium or potassium, an alkaline earth metal salt for example calcium or magnesium, or organic amine salt for example triethylamine.

- In vivo hydrolysable esters are those pharmaceutically acceptable esters that
- 25 hydrolyse in the human body to produce the parent compound. Such esters can be identified by administering, for example intravenously to a test animal, the compound under test and

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subsequently examining the test animal's body fluids. Suitable in vivo hydrolysable esters for carboxy include methoxymethyl and for hydroxy include acetyl.

In order to use a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof for the therapeutic treatment (including prophylactic treatment) of mammals including humans, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Therefore in another aspect the present invention provides a pharmaceutical composition which comprises a compound of the formula (I) or a pharmaceutically acceptable salt or an in vivo hydrolysable ester and pharmaceutically acceptable carrier.

The pharmaceutical compositions of this invention may be administered in standard manner for the disease condition that it is desired to treat, for example by oral, topical, parenteral, buccal, nasal, vaginal or rectal administration or by inhalation. For these purposes the compounds of this invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions, suspensions, emulsions, creams, ointments, gels, nasal sprays, suppositories, finely divided powders or aerosols for inhalation, and for parenteral use (including intravenous, intramuscular or infusion) sterile aqueous or oily solutions or suspensions or sterile emulsions.

In addition to the compounds of the present invention the pharmaceutical composition of this invention may also contain, or be co-administered (simultaneously or sequentially) with, one or more pharmacological agents of value in treating one or more disease conditions referred to hereinabove.

The pharmaceutical compositions of this invention will normally be administered to humans so that, for example, a daily dose of 0.5 to 75 mg/kg body weight (and preferably of 0.5 to 30 mg/kg body weight) is received. This daily dose may be given in divided doses as necessary, the precise amount of the compound received and the route of administration depending on the weight, age and sex of the patient being treated and on the particular disease condition being treated according to principles known in the art.

Typically unit dosage forms will contain about 1 mg to 500 mg of a compound of this invention.

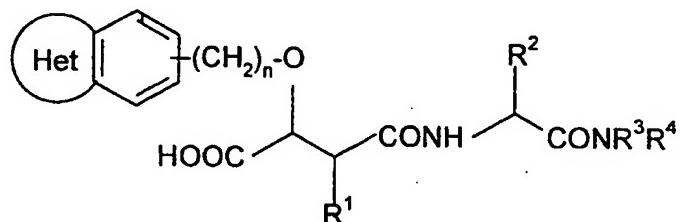
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Therefore in a further aspect, the present invention provides a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof for use in a method of therapeutic treatment of the human or animal body.

In yet a further aspect the present invention provides a method of treating a disease condition mediated by TNF which comprises administering to a warm-blooded animal an effective amount of a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof. The present invention also provides the use of a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof in the preparation of a medicament for use in a disease condition mediated by TNF.

10 In another aspect the present invention provides a process for preparing a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof which process comprises

a) reacting a compound of the formula (III):



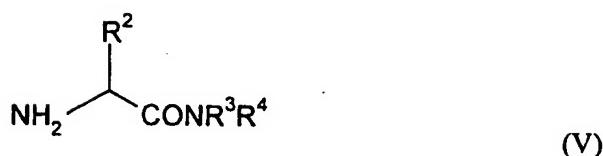
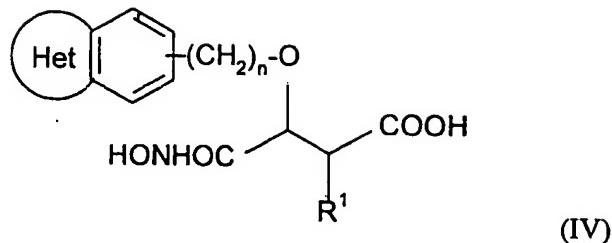
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(III)

wherein n, Het, R¹-R⁴ are as hereinbefore defined, or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine or a salt thereof; or

20 b) coupling a compound of the formula (IV) with a compound of the formula (V):

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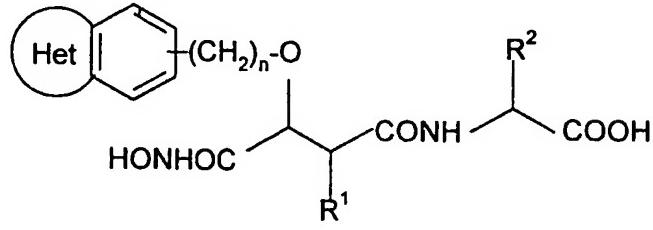


5

wherein n, Het, R¹-R⁴ are as hereinbefore defined, under standard peptide coupling conditions;
or

c) reacting a compound of the formula (VI) with compound of the formula (VII):

10



(VI)

15



wherein n, Het, R-R⁴ are as hereinbefore defined, under standard peptide coupling conditions:

wherein any functional group is protected, if necessary, and:

i. removing any protecting groups;

20 ii. optionally forming a pharmaceutically acceptable salt or in vivo hydrolysable ester.

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Protecting groups may in general be chosen from any of the groups described in the literature or known to the skilled chemist as appropriate for the protection of the group in question, and may be introduced by conventional methods.

Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting group in question, such methods being chosen so as to effect removal of the protecting group with minimum disturbance of groups elsewhere in the molecule.

Specific examples of protecting groups are given below for the sake of convenience, in which "lower" signifies that the group to which it is applied preferably has 1-4 carbon atoms. It will be understood that these examples are not exhaustive. Where specific examples of methods for the removal of protecting groups are given below these are similarly not exhaustive. The use of protecting groups and methods of deprotection not specifically mentioned is of course within the scope of the invention.

A carboxyl protecting group may be the residue of an ester-forming aliphatic or araliphatic alcohol or of an ester-forming silanol (the said alcohol or silanol preferably containing 1-20 carbon atoms).

Examples of carboxy protecting groups include straight or branched chain (1-12C)alkyl groups (eg isopropyl, *t*-butyl); lower alkoxy lower alkyl groups (eg methoxymethyl, ethoxymethyl, isobutoxymethyl); lower aliphatic acyloxy lower alkyl groups, (eg acetoxyethyl, propionyloxymethyl, butyryloxymethyl, pivaloyloxymethyl); lower alkoxycarbonyloxy lower alkyl groups (eg 1-methoxycarbonyloxyethyl, 1-ethoxycarbonyloxyethyl); aryl lower alkyl groups (eg benzyl, *p*-methoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, benzhydryl and phthalidyl); tri(lower alkyl)silyl groups (eg trimethylsilyl and *t*-butyldimethylsilyl); tri(lower alkyl)silyl lower alkyl groups (eg 25 trimethylsilylethyl); and (2-6C)alkenyl groups (eg allyl and vinylethyl).

Methods particularly appropriate for the removal of carboxyl protecting groups include for example acid-, base-, metal- or enzymically-catalysed hydrolysis.

Examples of hydroxyl protecting groups include lower alkyl groups (eg *t*-butyl), lower alkenyl groups (eg allyl); lower alkanoyl groups (eg acetyl); lower 30 alkoxycarbonyl groups (eg *t*-butoxycarbonyl); lower alkenyloxycarbonyl groups (eg

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allyloxycarbonyl); aryl lower alkoxy carbonyl groups (eg benzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *o*-nitrobenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl); tri lower alkylsilyl (eg trimethylsilyl, *t*-butyldimethylsilyl) and aryl lower alkyl (eg benzyl) groups.

- 5 Examples of amino protecting groups include formyl, aralkyl groups (eg benzyl and substituted benzyl, *p*-methoxybenzyl, nitrobenzyl and 2,4-dimethoxybenzyl, and triphenylmethyl); di-*p*-anisylmethyl and furylmethyl groups; lower alkoxy carbonyl (eg *t*-butoxycarbonyl); lower alkenyloxycarbonyl (eg allyloxycarbonyl); aryl lower alkoxy carbonyl groups (eg benzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *o*-nitrobenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl); trialkylsilyl (eg trimethylsilyl and *t*-butyldimethylsilyl); alkylidene (eg methylidene); benzylidene and substituted benzylidene groups.
- 10

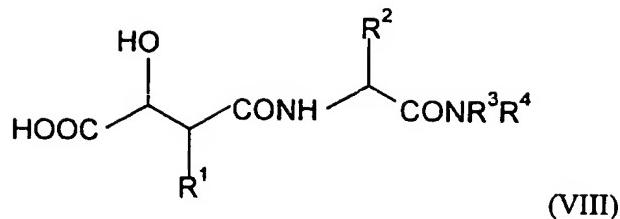
- Methods appropriate for removal of hydroxy and amino protecting groups include, for example, acid-, base-, metal- or enzymically-catalysed hydrolysis, for groups such as *p*-nitrobenzyloxycarbonyl, hydrogenation and for groups such as *o*-nitrobenzyloxycarbonyl, photolytically.

The hydroxylamine group (HONH-), in particular in process variants (b) and (c), is typically O-protected for example with benzyl, *t*-butyl or a silyl group.

- 15 The compound of the formula (III) may be reacted in the form of the acid or an activated derivative thereof such as an acid halide, acid anhydride or an 'activated' ester such as *1H*-benzo[1,2,3]triazol-1-yl, 1-hydroxy-benzo[1,2,3]triazole, pentafluorophenyl or 2,4,5-trichlorophenyl. The reaction of the compound of the formula (III) and hydroxylamine is performed under standard conditions. Typically the reaction of an activated ester of a compound of the formula (III) and hydroxylamine or O-protected hydroxylamine is performed
- 20
- 25 in the presence of a base, for example 2,6-lutidine in an anhydrous aprotic solvent, for example dimethylformamide, at a non-extreme temperature, for example in the region -30° to +25°, preferably about 0°C.

The compound of the formula (III) may be prepared by reacting a compound of the formula (VIII):

- 13 -



wherein R¹-R⁴ are as hereinbefore defined and preferably the carboxylic acid is protected:
with a compound of the formula (IX):

5



wherein L is a leaving group and n and Het are as hereinbefore defined.

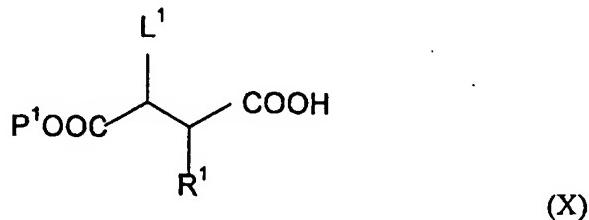
L is a leaving group for example halo such as chloro or bromo or a sulphonyloxy
10 group such as methanesulphonyloxy, trifluoromethanesulphonyloxy or p-
toluenesulphonyloxy.

Typically the reaction of the compounds (VIII) and (IX) is carried out in the
presence of a base, for example sodium hydride, in an anhydrous aprotic solvent for example
dimethylformamide or tetrahydrofuran, at a non-extreme temperature for example at ambient
15 temperature.

The compounds of the formula (IX) are prepared according to standard methods of
organic chemistry.

The compounds of the formula (VIII), preferably in carboxy-protected form, are
prepared by reacting a compound of the formula (V) with a compound of the formula (X):

20



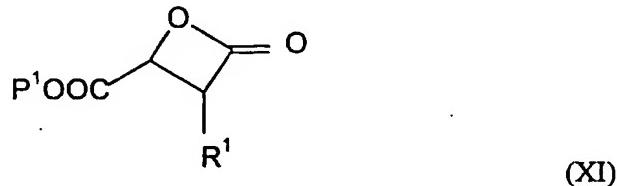
- 14 -

wherein P¹ is a carboxy-protecting group, R¹ is as hereinbefore defined and L¹ is a leaving group. Suitably L¹ is a leaving group such as halo, for example chloro, bromo or iodo, or a sulphonyloxy group, such as C₁₋₆alkanesulphonyloxy for example methanesulphonyloxy, benzenesulphonyloxy or 4-methylbenzenesulphonyloxy.

5 The reaction between the compounds of the formulae (V) and (X) is conveniently performed at a non-extreme temperature for example -25°C to +50°C and more conveniently 0°C to +30°C and most conveniently at ambient temperature.

The reaction is typically performed in a substantially inert organic solvent for example an aprotic solvent such as acetonitrile or diethyl ether.

10 The reaction of the compounds of the formulae (V) and (X) is believed to proceed via the formation of the lactone of the formula (XI).



15 wherein P¹ and R¹ are as hereinbefore defined.

The compound of the formula (V) acts as a base which is believed to convert the carboxylic acid function of the compound of the formula (X) to a carboxylate anion and which then displaces the leaving group L¹ to form the lactone. The lactone is believed to be ring-opened by nucleophilic attack of the compound of the formula (V) to form the compound 20 of the formula (VIII).

In another aspect a compound of the formula (XI) may be prepared by reacting a compound of the formula (X) with a non-nucleophilic base. In this way the base converts the carboxylic acid function of the compound of the formula (III) to a carboxylate anion which displaces L¹ to form the lactone. However the non-nucleophilic base does not substantially 25 react further with the lactone which may be isolated and then reacted with a compound of the formula (V).

Suitable non-nucleophilic bases include both organic and inorganic bases.

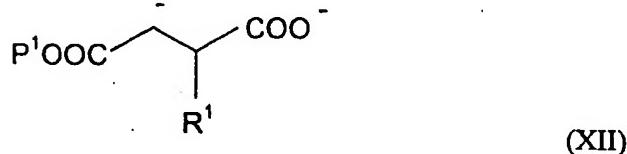
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- Preferably the base is an inorganic base such as an alkali metal or alkaline earth metal carbonate or bicarbonate for example sodium bicarbonate, potassium carbonate, sodium carbonate or potassium bicarbonate. Suitably the reaction is performed under biphasic conditions with the compound of the formula (X) dissolved in an aprotic organic solvent such
- 5 as acetonitrile, diethyl ether or dichloromethane which is stirred, typically vigorously, with an aqueous solution of the base at a non-extreme temperature for example at ambient temperature. The reaction may be monitored by thin layer chromatography or any other convenient methodology and, after a suitable period of time, the organic phase may be separated and worked-up to provide the compound of the formula (XI). In an alternative the
- 10 reaction is performed in the presence of a phase transfer catalyst for example benzyl trimethylammonium chloride, again with stirring at a non-extreme temperature.

In an alternative the non-nucleophilic base, for reacting with a compound of the formula (X), may be an organic base for example a tertiary amine such as di-isopropylethylamine. The reaction of a non-nucleophilic organic base with a compound of the

15 formula (X) is typically performed under standard conditions.

The compounds of the formula (X) may be prepared by reacting a dianion of the formula (XII):



20

wherein P^1 and R^1 are as hereinbefore defined, with a source of the group L^1 .

Suitable sources of halo include carbon tetrachloride and carbon tetrabromide.

Typically the dianion of the formula (XII) is formed by reacting the corresponding neutral compound with a non-nucleophilic base, for example lithium di-isopropylamide at low

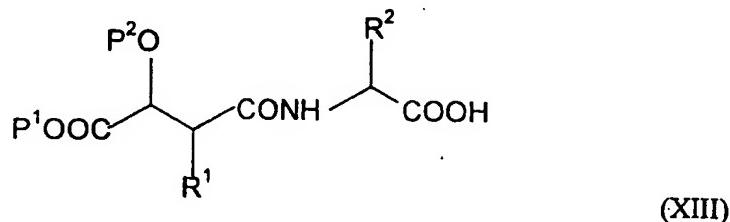
25 temperatures (-78°C) to form the dianion which is then reacted with the source of the group L^1 .

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The neutral compounds corresponding to the formula (XII) are known in, or may be made by, the methods of the literature.

In an alternative the compounds of the formula (VIII) may be prepared by reacting a compound of the formula (XIII) with a compound of the formula (VII):

5



wherein P¹, R¹-R⁴ are as hereinbefore defined and P² is a hydroxy protecting group under standard peptide coupling conditions and deprotecting as necessary. Conveniently P¹ and P² 10 may be linked, for example to form an acetal ring.

The compounds of the formula (XIII) may be prepared by reacting a compound of the formula (X) with an optionally carboxy-protected compound of the formula (XIV):

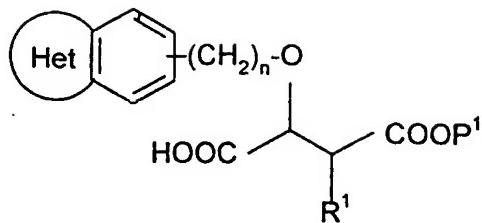


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wherein R² is as hereinbefore defined under standard peptide coupling conditions and subsequently protecting the carboxy group as necessary. The compound of the formula (X) may be formed in situ from the compound of the formula (XI).

The compounds of the formula (I) may also be prepared by reacting compounds of 20 the formulae (IV) and (V) as hereinbefore defined. This reaction is typically performed under standard peptide coupling conditions. The compounds of the formula (IV) may be prepared by reacting hydroxylamine, O-protected hydroxylamine or a salt thereof with a compound of the formula (XV):

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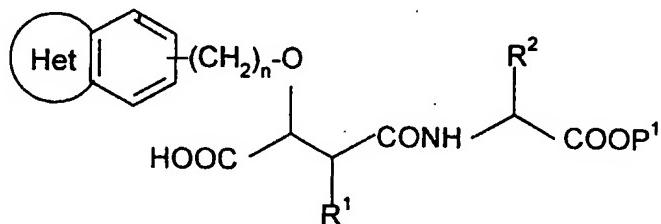


(XV)

- wherein P¹, R¹, n and Het are as hereinbefore defined, in a manner analogous to that described
 5 for reacting a compound of the formula (III), and thereafter deprotecting as necessary.

The compounds of the formula (I) may also be prepared by reacting compounds of the formulae (VI) and (VII) as hereinbefore defined, typically under standard peptide coupling conditions. The compounds of the formula (VI) may be prepared by reacting hydroxylamine, O-protected hydroxylamine or a salt thereof with a compound of the formula (XVI):

10



(XVI)

- 15 wherein P¹, n, R¹, R² and Het are as hereinbefore defined in a manner analogous to that described for reacting a compound of the formula (III) and subsequently deprotecting as necessary.

The compounds of the formula (XVI) may be prepared by reacting a hydroxy-deprotected compound of the formula (XIII) with a compound of the formula (IX).

- 20 The following biological test methods, data and Examples serve to illustrate the present invention.

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Isolated Enzyme Assay

The ability of the compounds of this invention to inhibit proTNF α convertase enzyme is assessed a partially purified, isolated enzyme assay, the enzyme being obtained from the membranes of THP-1 cells.

5 Assessment in human cell line (THF-2)

The ability of the compounds of this invention to inhibit TNF α production is assessed in THP-1 cells which are a human myelomonocytic cell line which synthesise and secrete TNF α when stimulated with lipopolysaccharide. THP-1 cells (4×10^5 cells in $160\mu\text{l}$ medium RPMI1640 + bicarbonate, penicillin, streptomycin and glutamine) are incubated 10 with $20\mu\text{l}$ of test compounds (triplicates) in DMSO or appropriate vehicle, in a 96 well tissue culture (TC) plate, for 30 min at 37°C in a humidified (5%CO₂/95%air) incubator, prior to addition of $20\mu\text{l}$ lipopolysaccharide (LPS) (E. coli. 0111:B4 (Sigma); final concentration $50\mu\text{g/ml}$). Each assay includes controls of THP-1 cells incubated with medium alone (six wells /plate) or with a standard TNF α inhibitor. The plates are then incubated for 6 hours at 37°C 15 (humidified incubator) after which time $100\mu\text{l}$ samples are removed from each well and transferred to a 96 well plate for storage at -70°C for subsequent analysis of TNF α concentration by ELISA. In this test, generally, compounds are of interest if they have activity below $10\mu\text{M}$.

Assessment in whole blood assay

20 The ability of the compounds of this invention to inhibit TNF α production is also assessed in a human whole blood assay (HWBA). Human whole blood secretes TNF α when stimulated with LPS. This property of blood forms the basis of an assay which is used as a secondary test for compounds which profile as active in the THP-1 test. Heparinized (10Units/ml) human blood obtained from volunteers is diluted 1:5 with medium (RPMI1640 25 + bicarbonate, penicillin, streptomycin and glutamine) and incubated ($160\mu\text{l}$) with $20\mu\text{l}$ of test compound (triplicates), in DMSO or appropriate vehicle, for 30 min at 37°C in a humidified (5%CO₂/95%air) incubator, prior to addition of $20\mu\text{l}$ LPS (E. coli. 0111:B4; final concentration $10\mu\text{g/ml}$). Each assay includes controls of diluted blood incubated with medium alone (6 wells/plate) or a known TNF α inhibitor as standard. The plates are then 30 incubated for 6 hours at 37°C (humidified incubator), centrifuged (2000rpm for 10 min; 4°C), plasma harvested ($50-100\mu\text{l}$) and stored in 96 well plates at -70°C before subsequent analysis.

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for TNF α concentration by ELISA. In this test, generally, compounds are of interest if they have activity below 50 μ M.

In vivo assessment

- The ability of the compounds of this invention as *ex vivo* TNF α inhibitors is
- 5 assessed in the rat. Briefly, groups of male Wistar Alderley Park (AP) rats (180-210g) are dosed with compound (6 rats) or drug vehicle (10 rats) by the appropriate route e.g. peroral (p.o.), intraperitoneal (i.p.), subcutaneous (s.c.). Ninety minutes later rats are sacrificed using a rising concentration of CO₂ and bled out via the posterior vena cavae into 5 Units of sodium heparin/ml blood. Blood samples are immediately placed on ice and centrifuged at 2000 rpm
 - 10 for 10 min at 4°C and the harvested plasmas frozen at -20°C for subsequent assay of their effect on TNF α production by LPS-stimulated human blood. The rat plasma samples are thawed and 175 μ l of each sample are added to a set format pattern in a 96U well plate. Fifty μ l of heparinized human blood is then added to each well, mixed and the plate is incubated for 30 min at 37°C (humidified incubator). LPS (25 μ l; final concentration 10 μ g/ml) is added to
 - 15 the wells and incubation continued for a further 5.5 hours. Control wells are incubated with 25 μ l of medium alone. Plates are then centrifuged for 10 min at 2000 rpm and 200 μ l of the supernatants are transferred to a 96 well plate and frozen at -20°C for subsequent analysis of TNF concentration by ELISA.
 - 20 Data analysis by dedicated software calculates for each compound/dose:
Percent inhibition =
$$\frac{\text{Mean TNF}\alpha \text{ (Controls)} - \text{Mean TNF}\alpha \text{ (Treated)}}{\text{Mean TNF}\alpha \text{ (Controls)}} \times 100$$

Pharmacodynamic test

- 25 To evaluate the clearance properties of the compounds of this invention a sensitive *ex vivo* pharmacodynamic test is employed which utilises the CON2 assay to evaluate clearance rate.

This is a generic test which can be used to estimate the clearance rate of compounds across a range of species. Animals (eg. rats, marmosets) are dosed iv with a soluble

- 30 formulation of compound and at subsequent time points (e.g. 5, 10, 15, 20, 30, 45, 60, 120 min) blood samples are taken from an appropriate vessel into 10U heparin. Plasma fractions

- 20 -

are obtained following centrifugation and the plasma proteins precipitated with ethanol (70% final concentration). After 30 mins at 4°C the plasma proteins are sedimented by centrifugation and the supernatant fraction is evaporated to dryness using a Savant speed vac. The sediment is reconstituted in CON2 assay buffer and subsequently analysed for compound 5 content using the TNF convertase assay (CON2). Briefly, a compound concentration-response curve is constructed for the compound undergoing evaluation. Serial dilutions of the reconstituted plasma extracts are assessed for activity and the amount of compound present in the original plasma sample is calculated using the concentration-response curve taking into account the total plasma dilution factor.

10

Test as anti-arthritis agent

Activity of a compound as an anti-arthritis is tested as follows. Acid soluble native type II collagen was shown by Trentham et al. [1] to be arthritogenic in rats: it caused polyarthritis when administered in Freunds incomplete adjuvant. This is now known as 15 collagen-induced arthritis (CIA) and similar conditions can be induced in mice and primates. Recent studies have shown that anti-TNF monoclonal antibodies [2] and TNF receptor-IgG fusion proteins [3] ameliorate established CIA indicating that TNF plays a key role in the pathophysiology of CIA. Moreover, the remarkable efficacy reported for anti-TNF 20 monoclonal antibodies in recent rheumatoid arthritis clinical trials indicate that TNF plays a major role in this chronic inflammatory disease. Thus CIA in DBA/1 mice as described in references 2 and 3 is a tertiary model which can be used to demonstrate the anti-arthritis activity of a compound.

1. Trentham, D.E. et al., (1977) J. Exp. Med., 146, 857.
- 25 2. Williams, R.O. et al., (1992) Proc Natl Acad Sci, 89, 9784.
3. Williams, R.O. et al., (1995) Immunology, 84, 433.

In the examples:

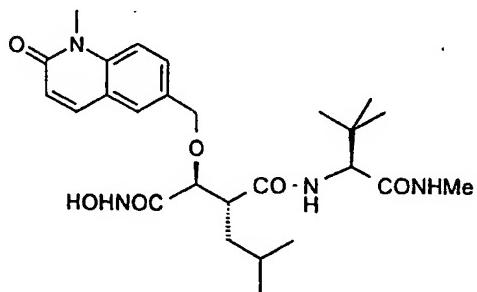
- 30 (a) NMR spectra were taken at 400 MHz;
(b) DMF means dimethylformamide;

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- (c) Evaporation of solvents was carried out under reduced pressure;
 - (d) LDA means lithium di-isopropylamide;
 - (e) THF means tetrahydrofuran;
 - (f) DMSO means dimethylsulphoxide;
- 5 (g) AcOH means acetic acid.

Example 1

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide



N²-[4-Hydroxy-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide (435 mg, 0.89 mmol) was dissolved in
 15 DMF (8 ml). 1-Hydroxybenzotriazole (180 mg, 1.35 mmol) was added, followed by N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (256 mg, 1.35 mmol), 2,6-lutidine (207 µl, 1.78 mmol) and O-(*tert*-butyl dimethylsilyl)hydroxylamine (180 mg, 1.16 mmol). The resulting solution was stirred at room temperature overnight. The crude reaction mixture was treated with HCl (2N, 1.5 ml) and purified by C18 preparative HPLC using as eluant a
 20 mixture of acetonitrile and water / 1 % AcOH (gradient from 1/9 to 35/65). Elution yielded the title compound (200 mg, 45 % yield) as a white solid : m.p. = 160-169°C ;
¹H-NMR (DMSO d₆) : 0.79 (d, 3H, J = 6.2 Hz), 0.81 (s, 9H), 0.85 (d, 3H, J = 6.2 Hz), 0.91 (m, 1H), 1.32-1.49 (m, 2H), 2.56 (d, 3H, J = 4.4 Hz), 2.95 (m, 1H), 3.62 (s, 3H), 3.80 (d, 1H, J = 9.5 Hz), 4.20 (d, 1H, J = 9.1 Hz), 4.32 (d, 1H, J = 11.7 Hz), 4.51 (d, 1H, J = 11.7 Hz), 6.64 (d, 1H, J = 9.1 Hz), 7.48 (m, 2H), 7.59 (s, 1H), 7.7 - 7.8 (m, 2H), 7.82 (d, 1H, J = 9.5 Hz), 9.11 (s, 1H), 10.87 (s, 1H) ; MS (ESI) : 525 (M + Na⁺) and 541 (M + K⁺).
 25

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N^2 -[4-Hydroxy-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide used as the starting material was obtained as follows :

- 5 (i) N^2 -[3S-Hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (400 mg, 1.07 mmol) was dissolved in THF (10 ml), under argon. Sodium hydride (50 mg, 60 % in oil, 1.2 mmol) was added followed 3 minutes later by a solution of 6-bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline^{Ref 1} (325 mg, 1.3 mmol) in THF (10 ml) and sodium iodide (161 mg, 1.07 mmol). The resulting mixture was stirred at room temperature overnight. The mixture was treated with a saturated solution of NH₄Cl and extracted with ethyl acetate. The combined organic extracts were dried over MgSO₄, filtered and the solvents were removed. The residue was purified by flash chromatography on silica using acetonitrile-dichloromethane (gradient from 3/7 to 3/2) as eluant to give N^2 -[2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (469 mg, 81 %) as a foam : MS (ESI) : 544 (M + H⁺) and 566 (M + Na⁺).
- 10 ii) Trifluoroacetic acid (1.38 ml) was added dropwise to a solution of N^2 -[2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (489 mg, 0.9 mmol) in dry dichloromethane (2 ml). The solution was stirred at room temperature overnight. The solvents were evaporated in vacuo. The residue was taken up in toluene and the solvent was removed in vacuo (three times). The residue was taken up in diethyl ether, triturated and filtered to give N^2 -[4-hydroxy-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide (440 mg, 100 %) : MS (ESI) : 488 (M + H⁺) and 510 (M + Na⁺).

N^2 -[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide used as starting material for step (i) was obtained as follows :

- 30 a) To a stirred solution of LDA [45.5 mmol ; prepared by addition of 2.5 M n-butyl lithium (18.2 ml , 45.5 mmol) in hexane to a solution of diisopropylamine (6.3 ml , 48.3

- 23 -

- mmol) in dry THF (20 ml) at -78°C] cooled to -78°C under argon was added dropwise a solution of 2R-isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester ^{Ref 2} (5.0 g , 21.7 mmol) in dry THF (15 ml). The mixture was stirred for 45 minutes at -78°C and a solution of carbon tetrachloride (2.3 ml , 23.9 mmol) in dry THF (3 ml) was added slowly, dropwise over ca. 8
- 5 minutes avoiding that the internal temperature rise above -65°C. The mixture was allowed to stir at -78°C for 30 minutes, warmed to room temperature and stirred for one hour at room temperature. The solution was cooled to -78°C and quenched by addition of HCl (2N, 3.3 ml). The solution was warmed to room temperature and extracted with diethyl ether. The combined organic extracts were dried over MgSO₄, filtered and the solvents were removed to
- 10 give directly one crude single isomer. The residue was purified by flash chromatography on silica using acetonitrile as eluant to give 3R-chloro-2S-isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester (5.6 g , 98 %) as a pale brown oil :
- ¹H-NMR (CDCl₃) : 0.94 (d, 3H, J = 4.8 Hz), 0.95 (d, 3H, J = 4.8 Hz), 1.48 (s, 9H), 1.55 (m, 1H), 1.65-1.8 (m, 2H), 3.05-3.1 (m, 1H), 4.41 (d, 1H, J = 8.1 Hz) ;
- 15 MS (EI) : 264 (M{³⁵Cl} + H⁺) and 266 (M{³⁷Cl} + H⁺).

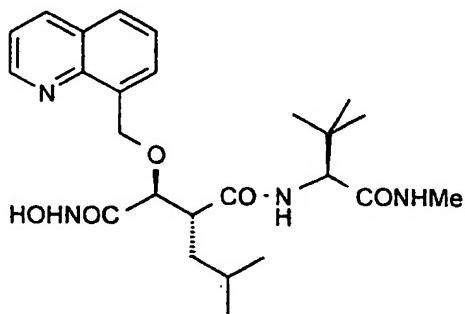
- b) To a stirred solution of 3R-chloro-2S-isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester (4.0 g , 15 mmol) in acetonitrile (100 ml) was added L-*tert*-leucine N-methylamide (2.8 g , 19.4 mmol). The mixture was stirred at room temperature for 24 hours. A further
- 20 quantity of acetonitrile (25 ml) was added and the mixture stirred for 12 hours. The solvents were evaporated in vacuo and the residue partitioned between water and ethyl acetate. The combined organic extracts were dried over MgSO₄, filtered and the solvents were removed. The residue was purified by flash chromatography on silica using acetonitrile-dichloromethane (gradient from 1/4 to 1/3) as eluant to give N²-[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (2.48 g , 45 %) as a beige solid :
- ¹H-NMR (CDCl₃) : 0.92 (d, 3H, J = 6.2 Hz), 0.96 (d, 3H, J = 6.2 Hz), 0.99 (s, 9H), 1.47 (s, 9H), 1.55-1.75 (m, 3H), 2.75 (m, 1H), 2.79 (d, 3H, J = 5.1 Hz), 3.73 (d, 1H, J = 5.9 Hz), 4.1 (m, 1H), 4.13 (d, 1H, J = 8.8 Hz), 5.88 (m, 1H), 6.68 (d, 1H, J = 9.1 Hz) ;
- MS (EI) : 373 (M + H⁺).
- 30 A small quantity of unreacted 3R-chloro-2S-isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester (336 mg) was recovered from the chromatography.

- 24 -

Example 2

N^2 -[4-(N-Hydroxyamino)-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide.

5



In a manner analogous to that described in the first paragraph of Example 1, from N^2 -[4-hydroxy-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide

- 10 (425 mg, 0.92 mmol) there was obtained the title compound (280 mg, 65 %) as a white solid : m.p. 118-121°C ;
¹H-NMR (DMSO d₆) : 0.80 (s, 9H), 0.81 (d, 3H, J = 6.2 Hz), 0.87 (d, 3H, J = 6.2 Hz), 1.0 (m, 1H), 1.37-1.52 (m, 2H), 2.55 (d, 3H, J = 4.8 Hz), 3.04 (m, 1H), 3.97 (d, 1H, J = 9.2 Hz), 4.17 (d, 1H, J = 9.2 Hz), 4.93 (d, 1H, J = 13.9 Hz), 5.14 (d, 1H, J = 13.9 Hz), 7.53-7.60 (m, 2H),
15 7.76-7.91 (m, 4H), 8.4 (m, 1H), 8.89 (m, 1H), 9.1 (s br, 1H), 11.2 (s br, 1H) ;
MS (ESI) : 473 (M + H⁺) and 495 (M + Na⁺).

The starting material was prepared as follows :

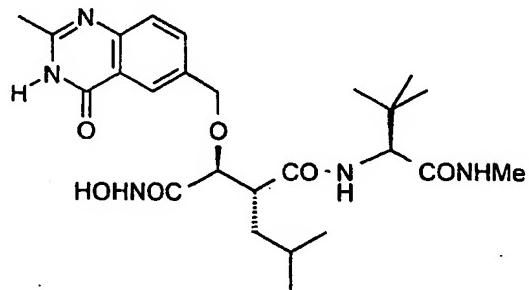
- 20 (i) In a manner analogous to that described in Example 1 (i), from N^2 -[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (500 mg, 1.34 mmol) and 8-iodomethylquinoline ^{Ref 3} (430 mg, 1.6 mmol) except that no sodium iodide was added, there was obtained N^2 -[2R-isobutyl-3S-(quinolin-8'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (540 mg, 79 %) as a gum:
25 MS (ESI) : 514 (M + H⁺) and 536 (M⁺Na⁺).

- 25 -

- (ii) In a manner analogous to that described in Example 1 (ii), from N²-[2R-isobutyl-3S-(quinolin-8'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (540 mg, 1.05 mmol) there was obtained N²-[4-hydroxy-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide (432 mg, 94.5 %) as a white solid:
- 5 MS (ESI) : 458 (M + H⁺) and 480 (M + Na⁺).

Example 3

- 10 N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide.



- In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2R-isobutyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxy succinyl]-L-*tert*-leucine-N¹-methylamide (470 mg, 0.96 mmol) there was obtained the title compound (152 mg, 32 %) as a white solid : m.p. = 180-187°C ;
- 15 ¹H-NMR (DMSO d₆) : 0.76 (s, 9H), 0.78 (d, 3H, J = 6.6 Hz), 0.84 (d, 3H, J = 6.6 Hz), 0.89 (m, 1H), 1.3-1.45 (m, 2H), 2.35 (s, 3H), 2.56 (d, 3H, J = 4.4 Hz), 2.97 (m, 1H), 3.81 (d, 1H, J = 9.5 Hz), 4.16 (d, 1H, J = 9.1 Hz), 4.34 (d, 1H, J = 11.7 Hz), 4.5 (d, 1H, J = 11.7 Hz), 7.49 (d, 1H, J = 8.4 Hz), 7.63 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.68-7.74 (m, 2H), 7.96 (d, 1H, J = 1.47 Hz), 9.12 (s br, 1H), 10.91 (s br, 1H), 12.16 (br, 1H) ;
- 20 MS (ESI) : 526 (M + Na⁺).

The starting material was prepared as follows :

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- (i) In a manner analogous to that described in Example 1 (i), from N²-[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (650 mg, 1.7 mmol) and 6-bromomethyl-2-methyl-4-oxo-3,4-dihydroquinazoline ^{Ref 4} (663 mg, 2.55 mmol) except that 15-crown-5 (1 drop) was also added, there was obtained N²-[2R-isobutyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (530 mg, 57 %) as a foam :
- MS (EI) : 545 (M + H⁺).

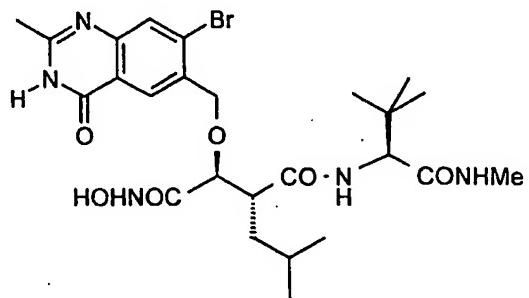
- (ii) In a manner analogous to that described in Example 1 (ii), from N²-[2R-isobutyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (530 mg, 0.97 mmol) there was obtained N²-[4-hydroxy-2R-isobutyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methyl amide (480 mg, 100 %) :
- MS (ESI) : 489 (M + H⁺) and 511 (M + Na⁺).

15

Example 4

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(7'-bromo-2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide.

20



- In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2R-isobutyl-3S-(7'-bromo-2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxy succinyl]-L-*tert*-leucine-N¹-methylamide (345 mg, 0.6 mmol) there was obtained the title compound (140 mg, 40 %) as a white powder :

m.p. = 184-188°C ;

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¹H-NMR (DMSO d₆) : 0.72 (s, 9H), 0.8 (d, 3H, J = 6.6 Hz), 0.86 (d, 3H, J = 6.6 Hz), 0.9 (m, 1H), 1.32-1.45 (m, 2H), 2.35 (s, 3H), 2.55 (d, 3H, J = 4.4 Hz), 3.12 (m, 1H), 3.9 (d, 1H, J = 9.9 Hz), 4.14 (d, 1H, J = 9.5 Hz), 4.44 (d, 1H, J = 12.8 Hz), 4.53 (d, 1H, J = 13.2 Hz), 7.7 (m, 1H), 7.78 (s, 1H), 7.83 (d, 1H, J = 9.15 Hz), 8.17 (s, 1H), 9.15 (s br, 1H), 11.0 (s br, 1H), 12.3 5 (s br 1H);

MS (ESI) : 582 (M{⁷⁹Br} + H⁺) and 584 (M{⁸¹Br} + H⁺).

The starting material was prepared as follows :

10 (i) In a manner analogous to that described in Example 1 (i), from N²-[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (470 mg, 1.26 mmol) and 7-bromo-6-bromomethyl-2-methyl-4-oxo-3,4-dihydroquinazoline (500 mg, 1.5 mmol) except that 15-crown-5 (1 drop) was also added, there was obtained N²-[2R-isobutyl-3S-(7'-bromo-2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (500 mg, 64 %) as a foam :

MS (ESI) : 645 (M{⁷⁹Br} + Na⁺) and 647 (M{⁸¹Br} + Na⁺).

(ii) In a manner analogous to that described in Example 1 (ii), from N²-[2R-isobutyl-3S-(7'-bromo-2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-20 *tert*-leucine-N¹-methylamide (500 mg, 0.8 mmol) there was obtained N²-[4-hydroxy-2R-isobutyl-3S-(7'-bromo-2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methyl amide (387 mg, 85%) :

MS (ESI) : 567 (M{⁷⁹Br} + H⁺) and 569 (M{⁸¹Br} + H⁺).

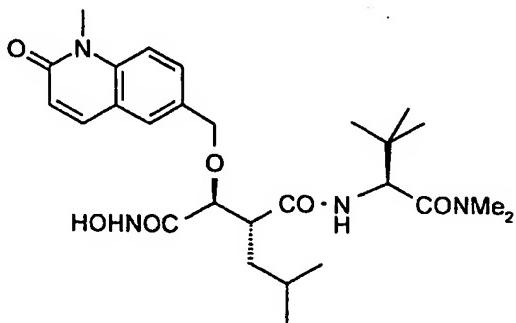
25 7-Bromo-6-bromomethyl-2-methyl-4-oxo-3,4-dihydroquinazoline was prepared by standard deprotection (aq. HCl) of (7-bromo-6-bromomethyl-2-methyl-4-oxo-3,4-dihydroquinazolin-3-yl)methyl 2,2-dimethylpropanoate [RN 140395-66-0; Zeneca Ltd.; (Pegg, S. J., Wardleworth, J. M.) UK Pat. Appl., GB 2264946 A1].

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Example 5

N^2 -[4-(N-Hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide.

5



In a manner analogous to that described in the first paragraph of Example 1, from N^2 -[4-hydroxy-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy succinyl]-L-*tert*-leucine-N¹-dimethylamide (205 mg, 0.4 mmol) there was obtained the title compound

10 (116 mg, 56 %) as a white solid :

m.p. = 160-166°C ;

1H -NMR (DMSO d₆) : 0.78 (d, 3H, J = 6.6 Hz), 0.83 (d, 3H, J = 6.6 Hz), 0.86 (s, 9H), 0.9 (m, 1H), 1.3-1.45 (m, 2H), 2.81 (s, 3H), 2.99 (m, 1H), 3.08 (s, 3H), 3.63 (s, 3H), 3.81 (d, 1H, J = 9.9 Hz), 4.33 (d, 1H, J = 11.7 Hz), 4.51 (d, 1H, J = 11.7 Hz), 4.76 (d, 1H, J = 9.2

15 Hz), 6.64 (d, 1H, J = 9.5 Hz), 7.5 (m, 2H), 7.6 (s, 1H), 7.84 (m, 2H), 9.1 (s br, 1H), 10.89 (s br, 1H) ; MS (ESI) : 517 (M + H⁺) and 539 (M + Na⁺).

The starting material was prepared as follows :

- 20 (i) In a manner analogous to that described in Example 1 (i), from N^2 -[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide (197 mg, 0.51 mmol) and 6-bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline (141 mg, 0.56 mmol) there was obtained N^2 -[2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide (243 mg, 85%) as a gum :
- 25 MS (EI) : 558 (M + H⁺) and 580 (M + Na⁺).

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- (ii) In a manner analogous to that described in Example 1 (ii), from N^2 -[2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide (242 mg, 0.43 mmol) there was obtained N^2 -[4-hydroxy-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide (210 mg, 97 %) as a white solid: MS (ESI) : 502 ($M + H^+$) and 524 ($M + Na^+$).

N^2 -[3S-Hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide used as starting material for step (i) was obtained as follows :

- 10 In a manner analogous to that described in Example 1 (b), to a solution of 3R-chloro-2S-isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester (500 mg, 1.89 mmol) in acetonitrile (7 ml) was added L-*tert*-leucine N-dimethylamide^{NOTE} (448 mg, 2.83 mmol). The mixture was stirred at room temperature for 24 hours. The mixture was poured into aqueous NH_4Cl (10%) and extracted with ethyl acetate. The combined organic extracts were washed with water, dried over $MgSO_4$, filtered and the solvents were removed. The residue was purified by flash chromatography on silica using acetonitrile-dichloromethane (1/4) as eluant to give N^2 -[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide (395 mg, 54 %) as a gum which solidified :
- 15

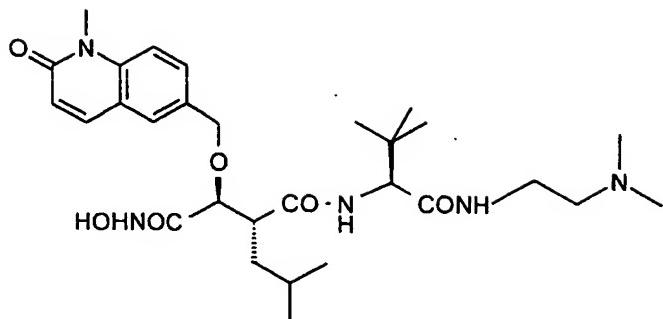
¹H-NMR ($CDCl_3$) : 0.92 (d, 3H, $J = 5.5$ Hz), 0.95 (d, 3H, $J = 5.8$ Hz), 0.98 (s, 9H), 1.47 (s, 9H), 1.55-1.7 (m, 3H), 2.72 (m, 1H), 2.95 (s, 3H), 3.1 (s, 3H), 3.86 (s br, 1H), 4.07 (m, 1H), 4.86 (d, 1H, $J = 9.5$ Hz), 6.61 (d, 1H, $J = 9.2$ Hz) ; MS (EI) : 409 ($M + Na^+$).

^{NOTE} L-*tert*-leucine N-dimethylamide was prepared by the reaction of L-*tert*-leucine with triphosgene to give 3-(S)-*tert*-butyl oxazolidine-1,4-dione which was then treated with a saturated ethereal solution of dimethylamine.

Example 6

N^2 -[4-(N-Hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-(2-dimethylaminoethyl)amide.

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In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy succinyl]-L-tert-leucine-N¹-(2-dimethylaminoethyl)amide (230 mg, 0.35 mmol) there was obtained the
5 title compound (60 mg, 50 %) as a white solid :

m.p. = 95-102°C ;
¹H-NMR (DMSO d₆ + TFA) : 0.79 (d, 3H, J = 6.4 Hz), 0.84 (s, 9H), 0.86 (d, 3H, J = 6.6 Hz),
1.0 (m, 1H), 1.41 (m, 2H), 2.81 (s, 6H) 3.0 - 3.2 (m, 3H), 3.3 - 3.45 (m, 2H), 3.62 (s, 3H), 3.8
(d, 1H, J = 9.5 Hz), 4.11 (m, 1H), 4.4 (m, 2H), 6.64 (d, 1H J = 9.5 Hz), 7.45 - 7.9
10 (m, 4H), 8.2 (m, 2H), 9.4 (s br, 1H). MS (ESI) : 560 (M + H⁺).

The starting material was prepared as follows :

- (i) In a manner analogous to that described in Example 1 (i), from N²-[3S-hydroxy-2R-
15 isobutyl-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-(2-dimethylaminoethyl)amide (220 mg,
0.51 mmol) and 6-bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline (142 mg, 0.56 mmol)
there was obtained N²-[2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-
yl)methoxy-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-(2-dimethylaminoethyl)amide (132
mg, 43%): MS (EI) : 601 (M + H⁺).
20 (ii) In a manner analogous to that described in Example 1 (ii), from N²-[2R-isobutyl-3S-(1'-
methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy-4-tert-butyloxysuccinyl]-L-tert-leucine-
N¹-(2-dimethylaminoethyl)amide (230 mg, 0.38 mmol) there was obtained N²-[4-hydroxy-2R-
isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-tert-leucine-
N¹-(2-dimethylaminoethyl)amide (240 mg, 96 %) as a hygroscopic solid:
25 MS (ESI) : 545 (M + H⁺).

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N²-[3S-Hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-(2-dimethylaminoethyl)amide used as starting material for step (i) was obtained as follows:

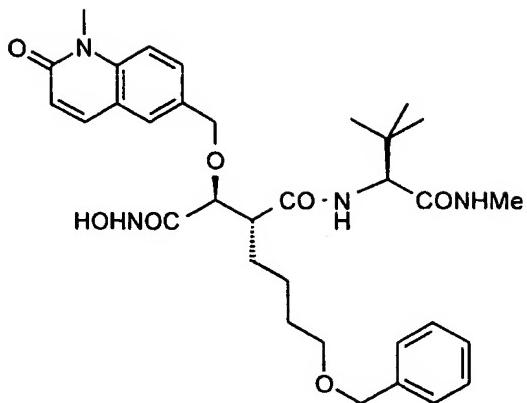
In a manner analogous to that described in Example 1 (b), to a solution of 3R-5 chloro-2S-isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester (500 mg, 1.89 mmol) in acetonitrile (7 ml) was added L-*tert*-leucine N-(2-dimethylaminoethyl)amide^{NOTE} (455 mg, 2.2 mmol). The mixture was stirred at room temperature for 48 hours. The mixture was poured into aqueous NH₄Cl (10%) and extracted with ethyl acetate. The combined organic extracts were washed with water, dried over MgSO₄, filtered and the solvents were removed. The residue 10 was purified by flash chromatography on silica using methanol-dichloromethane (1/9) as eluant to give N²-[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-(2-dimethylaminoethyl)amide(437 mg, 54 %) as a gum:
¹H-NMR (CDCl₃) : 0.93 (d, 3H, J = 5.8 Hz), 0.96 (d, 3H, J = 6.2 Hz), 1.0 (s, 9H), 1.48 (s, 9H), 1.55-1.71 (m, 3H), 2.23 (s, 6H), 2.46 (m, 2H), 2.76 (m, 1H), 3.34 (m, 2H), 4.11 (d, 1H, J = 3.3 Hz), 4.19 (d, 1H, J = 9.2 Hz), 6.6 (m, 2H);
MS (EI) : 430 (M + H⁺) and 452 (M + Na⁺).

^{NOTE} L-*tert*-leucine 2-dimethylaminoethylamide was prepared by the reaction of L-*tert*-leucine with triphosgene to give 3-(S)-*tert*-butyl oxazolidine-1,4-dione which was then treated with 20 N,N-dimethyl ethylenediamine.

Example 7

N²-[4-(N-Hydroxyamino)-2R-(4'-benzyloxy)butyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide.

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In a manner analogous to that described in the first paragraph of Example 1, from N^2 -[4-hydroxy-2R-(4'-benzyloxy)butyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide (300 mg, 0.5 mmol) there was obtained the title compound (145 mg, 47 %) as a white solid : m.p. = 188-190°C ;
 1H -NMR ($CDCl_3$) : 0.86 (s, 9H), 1.39-1.45 (m, 2H), 1.5-1.8 (m, 4H), 2.89 (d, 3H, J = 4.39 Hz), 3.05-3.1 (m, 1H), 3.41 (t, 2H, J = 6.23 Hz), 3.7 (s, 3H), 3.94 (d, 1H, J = 9.2 Hz), 4.1 (d, 1H, J = 3.3 Hz), 4.45 (d, 2H, J = 1.5 Hz), 4.5 (d, 1H, J = 11.0 Hz), 4.96 (d, 1H, J = 11.0 Hz), 6.72 (d, 1H, J = 9.5 Hz), 6.95-7.0 (m, 1H), 7.25-7.38 (m, 6H), 7.55 (d, 1H, J = 8.8 Hz), 7.61 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.65 (d, 1H, J = 9.5 Hz), 7.69 (d, 1H, J = 1.5 Hz), 9.6 (s br, 2H) ;
MS (ESI) : 609 ($M + H^+$) and 631 ($M + Na^+$).

The starting material was prepared as follows :

15

(i) In a manner analogous to that described in Example 1 (i), from N^2 -[3S-hydroxy-2R-(4'-benzyloxy)butyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (480 mg, 1.0 mmol) and 6-bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline (278 mg, 1.1 mmol) there was obtained N^2 -[2R-(4'-benzyloxy)butyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (402 mg, 61%) as a white solid: MS (EI) : 650 ($M + H^+$) and 672 ($M + Na^+$).

(ii) In a manner analogous to that described in Example 1 (ii), from N^2 -[2R-(4'-benzyloxy)butyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxy-4-*tert*-

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butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (380 mg, 0.58 mmol) there was obtained N²-[4-hydroxy-2R-(4'-benzyloxy)butyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide (335 mg, 97 %) as a fine white powder: MS (ESI) : 594 (M + H⁺) and 616 (M + Na⁺).

5

N²-[3S-Hydroxy-2R-(4'-benzyloxy)butyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide used as starting material for step (i) was obtained as follows:

In a manner analogous to that described in Example 1 (a), to a stirred solution of
 10 LDA [11.24 mmol; prepared by addition of 2.5 M n-butyl lithium (4.5 ml, 11.24 mmol) in hexane to a solution of diisopropylamine (1.57 ml, 11.24 mmol) in dry THF (4 ml) at -78°C] cooled to -78°C under argon was added dropwise a solution of 2R-(4'-benzyloxy)butyl-butan-1,4-dioic acid-4-*tert*-butyl ester ^{Ref 5} (1.8 g, 5.35 mmol) in dry THF (2 ml). The mixture was stirred for 70 minutes at -78°C and a solution of carbon tetrachloride (0.566 ml, 5.89 mmol) in
 15 dry THF (1 ml) was added slowly, dropwise over ca. 8 minutes avoiding that the internal temperature rise above -65°C. The mixture was allowed to stir at -78°C for 60 minutes and quenched by addition of HCl (2N). The solution was warmed to room temperature and extracted with diethyl ether. The combined organic extracts were dried over MgSO₄, filtered and the solvents were removed to give directly crude 3R-chloro-2S-(4'-benzyloxy)butyl-
 20 butan-1,4-dioic acid-4-*tert*-butyl ester (2.13 g, 100 %) as a brown oil used as such in the following step:

¹H-NMR (CDCl₃) : 1.3-1.75 (m, 6H), 1.47 (s, 9H), 3.04 (m, 1H), 3.47 (t, 2H, J = 6.3 Hz), 4.37 (d, 1H, J = 9.1 Hz), 4.49 (s, 2H), 7.29-7.34 (m, 5H);
 MS (EI) : 371 (M{³⁵Cl} + H⁺) and 373 (M{³⁷Cl} + H⁺).

25 In a manner analogous to that described in Example 1 (b), to a solution of 3R-chloro-2S-(4'-benzyloxy)butyl-butan-1,4-dioic acid-4-*tert*-butyl ester (1.9 g, 5.12 mmol) in acetonitrile (40 ml) was added L-*tert*-leucine N-methylamide (738 mg, 6.15 mmol). The mixture was stirred at room temperature for 16 hours. The mixture was poured into aqueous NH₄Cl (10%) and extracted with ethyl acetate. The combined organic extracts were washed
 30 with water, dried over MgSO₄, filtered and the solvents were removed. The residue was purified by flash chromatography on silica using acetonitrile-dichloromethane (gradient from

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3/17 to 7/13) as eluant to give N^2 -[3S-hydroxy-2R-(4'-benzyloxy)butyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (520 mg, 27 %) as a pale brown gum :
¹H-NMR (CDCl₃) : 0.98 (s, 9H), 1.46 (s, 9H), 1.6-1.9 (m, 6H), 2.66 (m, 1H), 2.77 (d, 3H, J = 4.8 Hz), 3.46 (t, 2H, J = 6.3 Hz), 3.74 (s br, 1H), 4.12 (m, 2H), 4.49 (m, 2H), 5.79 (m, 1H), 6.71 (d, 1H, J = 9.2 Hz) 7.33-7.36 (m, 5H);
MS (EI) : 479 (M + H⁺).

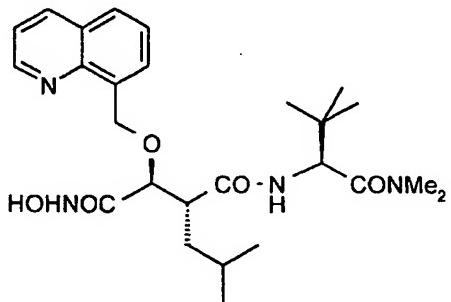
A quantity of unreacted 3R-chloro-2S-(4'-benzyloxy)butyl-butan-1,4-dioic acid-4-*tert*-butyl ester (412 mg) was recovered from the chromatography.

10

Example 8

N^2 -[4-(N-Hydroxyamino)-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide

15



In a manner analogous to that described in the first paragraph of Example 1, from N^2 -[4-hydroxy-2R-isobutyl-3S-(quinolin-8'-yl)methoxy succinyl]-L-*tert*-leucine-N¹-dimethylamide (150 mg, 0.31 mmol) there was obtained the title compound (48 mg, 32 %) as a white solid :

20 m.p. = 145-151°C ;

¹H-NMR (DMSO d₆) : 0.78 (d, 3H, J = 6.6 Hz), 0.83 (s, 9H), 0.84 (d, 3H), 1.0 (m, 1H), 1.35-1.5 (m, 2H), 2.77 (s, 3H), 3.04 (s, 3H), 3.06 (m, 1H), 3.94 (d, 1H, J = 9.5 Hz), 4.72 (d, 1H, J = 9.2 Hz), 4.90 (d, 1H), 5.13 (d, 1H), 7.5-7.6 (m, 2H), 7.8-7.95 (m, 3H), 8.38 (m, 1H), 8.89 (m, 1H), 9.1 (s, 1H), 11.18 (s, 1H) ;

25 MS (ESI) : 487 (M + H⁺) and 509 (M + Na⁺).

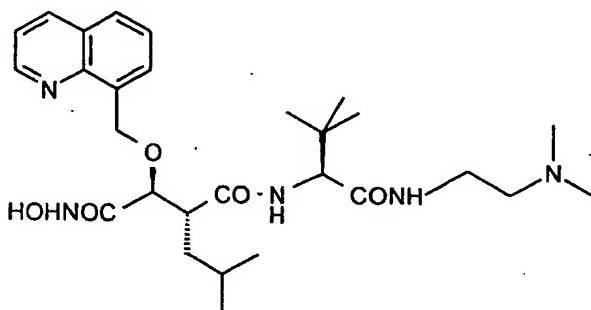
- 35 -

The starting material was prepared as in Example 5 using 8-iodomethylquinoline as alkylating agent.

Example 9

5

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-tert-leucine-N¹-(2-dimethylaminoethyl)amide

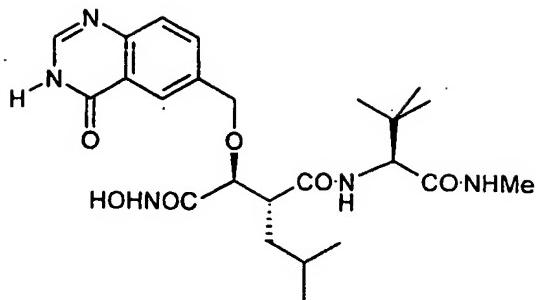


- 10 In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-tert-leucine-N¹-(2-dimethylaminoethyl)amide (384 mg, 0.5 mmol) there was obtained the title compound (115 mg, 39 %) as a white solid : m.p. = 85-90°C ;
¹H-NMR (DMSO d₆) : 0.79 (d, 3H), 0.80 (s, 9H), 0.86 (d, 3H, J = 6.2 Hz), 0.97 (m, 1H), 1.35-15 1.55 (m, 2H), 3.0 - 3.3 (m, 5H), 3.32 (s, 6H), 3.95 (d, 1H, J = 9.5 Hz), 4.16 (d, 1H, J = 8.8 Hz), 4.91 (d, 1H), 5.12 (d, 1H), 7.5 - 7.6 (m, 2H), 7.8 - 7.9 (m, 4H), 8.38 (d, 1H, J = 6.6 Hz), 8.88 (dd, 1H, J = 1.8 Hz, J = 4.0 Hz), 9.1 (s br, 1H) 11.2 (s br, 1H);
MS (ESI) : 530 (M + H⁺).

The starting material was prepared as in Example 6 using 8-iodomethylquinoline as alkylating agent.

Example 10

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide



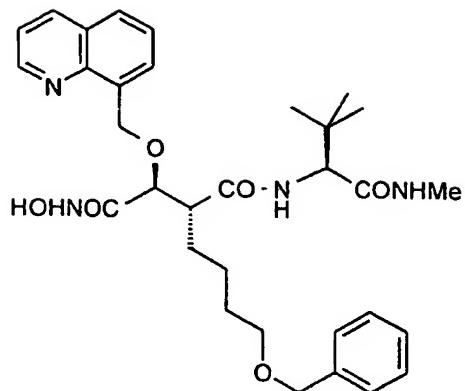
In a manner analogous to that described in the first paragraph of Example 1, from *N*²-[4-hydroxy-2*R*-isobutyl-3*S*-(4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-*L*-*tert*-leucine-*N*¹-methylamide (60 mg, 0.12 mmol) there was obtained the title compound (20 mg, 34 %) as a white amorphous solid : m.p. = 162-165°C ;
¹H-NMR (DMSO d₆) : 0.74 (s, 9H), 0.77 (d, 3H, J = 6.6 Hz), 0.83 (d, 3H, J = 6.2 Hz), 0.89 (m, 1H), 1.3-1.5 (m, 2H), 2.53 (d, 3H, J = 4.4 Hz), 3.0 (m, 1H), 3.8 (d, 1H, J = 9.9 Hz), 4.15 (d, 1H, J = 9.5 Hz), 4.36 (d, 1H), 4.53 (d, 1H), 7.58 (d, 1H, J = 8.4 Hz), 7.65 - 7.75 (m, 3H), 8.0 10 (d, 1H, J = 1.8 Hz), 8.06 (s, 1H), 9.1 (s br, 1H), 11.1 (s br, 1H), 11.8 (s br, 1H);
MS (ESI) : 490 (M + H⁺) and 512 (M + Na⁺).

The starting material was prepared as in Example 3, using as alkylating agent, 6-bromomethyl-4-oxo-3,4-dihydroquinazoline (prepared by bromination (NBS, AIBN in refluxing CCl₄) of 6-15 methylquinazolin-4-one (RN 19181-53-4; commercially available from Maybridge).

Example 11

*N*²-[4-(*N*-Hydroxyamino)-2*R*-(4'-benzyloxy)butyl-3*S*-(quinolin-8'-yl)methoxysuccinyl]-*L*-20 *tert*-leucine-*N*¹-methylamide

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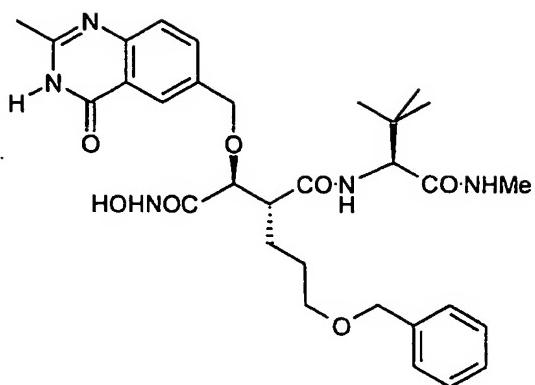


In a manner analogous to that described in the first paragraph of Example 1, from N^2 -[4-hydroxy-2R-(4'-benzyloxy)butyl-3S-(quinolin-8'-yl)methoxy succinyl]-L-tert-leucine-N¹-methylamide (250 mg, 0.44 mmol) there was obtained the title compound (110 mg, 43 %) as a
 5 white solid : m.p. = 92-94°C ;
¹H-NMR (CDCl₃) : 0.64 (s, 9H), 1.4-1.46 (m, 2H), 1.58-1.66 (m, 2H), 1.73-1.79 (m, 2H), 2.74 (d, 3H, J = 4.88 Hz), 2.84-2.89 (m, 1H), 3.44 (t, 2H, J = 6.35 Hz), 3.99 (d, 1H, J = 9.1 Hz), 4.34 (d, 1H, J = 4.6 Hz), 4.46 (s, 2H), 4.68 (d, 1H), 5.43 (d, 1H), 6.08 (s br, 1H), 6.85 (d, 1H, J = 8.8 Hz), 7.31 (m, 5H), 7.51-7.56 (m, 2H), 7.68 (dd, 1H, J = 7.1 Hz, J = 1.2 Hz), 7.88 (dd, 10 1H, J = 8.2 Hz, J = 1.1 Hz), 8.27 (dd, 1H, J = 8.3 Hz, J = 1.7 Hz), 9.06 (dd, 1H, J = 4.4 Hz, J = 1.7 Hz), 14.2 (s, 2H) ;
 MS (ESI) : 579 (M + H⁺).

The starting material was prepared as in Example 7 using 8-iodomethylquinoline as alkylating
 15 agent.

Example 12

N^2 -[4-(N-Hydroxyamino)-2R-(3'-benzyloxy)propyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.



In a manner analogous to that described in the first paragraph of Example 1, except that O-(2,4-dimethoxybenzyl)hydroxylamine was used instead of O-(*tert*-butyldimethylsilyl)hydroxylamine and the reaction mixture was treated with 5% TFA/dichloromethane instead of HCl (2N), from N²-[4-hydroxy-2*R*-(3'-benzyloxy)propyl-3*S*-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide (1.0 g, 1.7 mmol) there was obtained the title compound (430 mg, 41 %) as a white solid : m.p. = 189-194°C ;

10 ¹H-NMR (DMSO d₆) : 0.77 (s, 9H), 1.2-1.5 (m, 4H), 2.33 (s, 3H), 2.53 (d, 3H, J = 4.4 Hz), 2.93 (m, 1H), 3.32 (m, 2H), 3.86 (d, 1H, J = 9.9 Hz), 4.17 (d, 1H, J = 9.2 Hz), 4.34 (d, 1H), 4.40 (s, 2H), 4.5 (d, 1H), 7.25-7.36 (m, 5H), 7.47 (d, 1H, J = 8.4 Hz), 7.61 (d, 1H, J = 8.1 Hz), 7.79 (m, 2H), 7.93 (s, 1H), 9.1 (s, 1H), 10.9 (s, 1H), 14.2 (s, 1H) ;
 MS (ESI) : 596 (M + H⁺) and 618 (M + Na⁺).

15

The starting material was prepared in a manner analogous to Example 7 using 6-bromomethyl-2-methyl-4-oxo-3,4-dihydroquinazoline as alkylating agent.
 The 3*R*-chloro-2*S*-(3'-benzyloxy)propyl-butan-1,4-dioic acid-4-*tert*-butyl ester was prepared from 2*R*-(3'-benzyloxy)propan-butan-1,4-dioic acid-4- *tert*-butyl ester ^{Ref 5} in a manner
 20 analogous to Example 7.

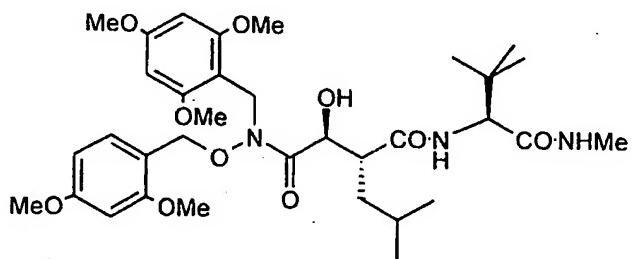
Examples 13-22**(General Procedure)**

For Examples 13-22 the final two steps were accomplished, as described specifically in

- 5 Example 13, by alkylation and subsequent deprotection of N²-[4-(N-2',4'-dimethoxybenzyloxy-N-2',4',6'-trimethoxybenzylamino)-3S-hydroxy-2R-isobutylsuccinyl]-L-*tert*-leucine-N¹-methylamide which was obtained as follows from N²-[3S-Hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide [described in Example 1, paragraphs a) and b)].

10

N²-[4-(N-2',4'-Dimethoxybenzyloxy-N-2',4',6'-trimethoxybenzylamino)-3S-hydroxy-2R-isobutylsuccinyl]-L-*tert*-leucine-N¹-methylamide



15

- i) Trifluoroacetic acid (40 ml) was added dropwise to a solution of N²-[3S-hydroxy-2R-isobutyl-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methylamide (10.2 g, 27.4 mmol) in dry dichloromethane (40 ml). The solution was stirred at room temperature overnight. The solvents were evaporated in vacuo. The residue was taken up in toluene and the solvent was removed in vacuo (three times). The residue was taken up in diethyl ether, triturated and filtered to give N²-[3S 3,4-dihydroxy-2R-isobutylsuccinyl]-L-*tert*-leucine-N¹-methylamide (8.6 g, 100 %) : MS (ESI) : 317 (M + H⁺) and 339 (M + Na⁺).
- ii) N²-[3S 3,4-dihydroxy-2R-isobutylsuccinyl]-L-*tert*-leucine-N¹-methylamide (8.0 g, 25.3 mmol) was dissolved in DMF (150 ml). 1-Hydroxybenzotriazole (5.1 g, 37.9 mmol) was added, followed by N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (7.2 g, 37.9 mmol), 2,6-lutidine (588 µl, 5 mmol) and O-(2,4-dimethoxybenzyl-N-2,4,6-trimethoxybenzyl)hydroxylamine (10.1 g, 27.8 mmol). The resulting solution was stirred at

- 40 -

room temperature overnight. The crude reaction mixture was concentrated and partitioned between water and ethyl acetate. The combined organic extracts were washed with HCl (1N), brine, dried over MgSO₄, filtered and the solvents were removed. The residue was purified by flash chromatography on silica using acetonitrile-dichloromethane (2/3) as eluant to give the

5 title compound (8.8 g , 53 % yield) as a white solid :

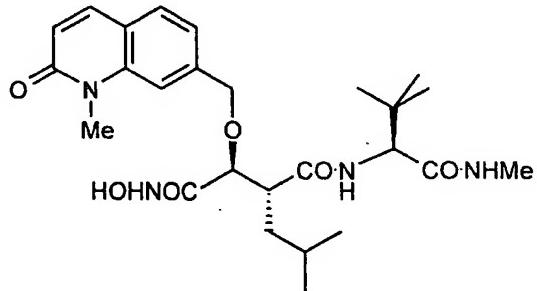
¹H-NMR (CDCl₃) : 0.7 (d, 3H, J = 6.2 Hz), 0.81 0.8 (d, 3H, J = 6.2 Hz), 1.04 (s, 9H), 1.37-1.64 (m, 3H), 2.69 (m, 1H), 2.79 (d, 3H, J = 4.8 Hz), 3.37 (d, 1H, J = 4.0 Hz), 3.76 (s, 6H), 3.79 (s, 6H), 3.83 (s, 3H), 4.12 (d, 1H, J = 8.4 Hz), 4.52 (m, 1H), 4.81 (m, 2H), 4.89 (d, 1H, J = 13.9 Hz), 5.14 (d, 1H, J = 14.2 Hz), 6.14 (s, 2H), 6.4 (m, 2H), 6.85 - 7.0 (m, 3H).

10

Example 13

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-7'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide

15



a) N²-[4-(N-2',4'-Dimethoxybenzyloxy-N-2',4',6'-trimethoxybenzylamino)-3S-hydroxy-2R-isobutylsuccinyl]-L-tert-leucine-N¹-methylamide (330 mg, 0.5 mmol) was dissolved in dry THF (10 ml), under argon. Sodium hydride (23 mg, 60 % in oil, 0.57 mmol) was added followed 30 minutes later by 7-bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline ^{Ref 6} (138 mg, 0.54 mmol), sodium iodide (75 mg, 0.5 mmol) and one drop of 15-crown-5. The resulting mixture was stirred at room temperature overnight. The mixture was treated with a saturated solution of NH₄Cl and extracted with ethyl acetate. The combined organic extracts were dried over MgSO₄, filtered and the solvents were removed. The residue was purified by flash chromatography on silica using acetonitrile-dichloromethane (gradient from 1/2 to 1/1)

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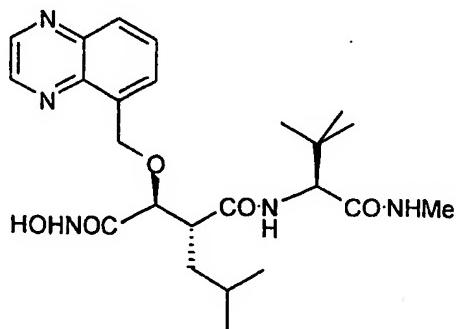
as eluant to give N²-[4-(N-2',4'-dimethoxybenzyloxy-N-2',4',6'-trimethoxybenzylamino)-2R-isobutyl- 3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-7'-yl)succinyl]-L-*tert*-leucine-N¹-methylamide (334 mg, 80 %) : MS (ESI) : 833 (M + H⁺) and 855 (M + Na⁺).

5 b) Trifluoroacetic acid (0.8 ml) was added dropwise to a solution of N²-[4-(N-2',4'-dimethoxybenzyloxy-N-2',4',6'-trimethoxybenzylamino)-2R-isobutyl- 3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-7'-yl)succinyl]-L-*tert*-leucine-N¹-methylamide (300 mg, 0.36 mmol) in dry dichloromethane (7.2 ml). The solution was stirred at room temperature for 12 hours. The solvents were evaporated in vacuo. The residue was taken up in toluene and the solvent was
 10 removed in vacuo (three times). The residue was taken up in methanol, triturated and filtered to give the crude product which was purified by C18 preparative HPLC using as eluant a mixture of methanol and water / 1 % AcOH (1/1) to give the title compound (70 mg, 38 %) as a white solid. m.p. = 176-178°C ;

¹H-NMR (DMSO d₆) : 0.76 (d, 3H, J = 6.6 Hz), 0.81 (s, 9H), 0.83 (d, 3H, J = 6.6 Hz), 0.9 (m, 1H), 1.4-1.6 (m, 2H), 2.54 (d, 3H, J = 4.4 Hz), 2.95 (m, 1H), 3.63 (s, 3H), 3.81 (d, 1H, J = 9.9 Hz), 4.20 (d, 1H, J = 9.2 Hz), 4.4 (d, 1H), 4.6 (d, 1H), 6.59 (d, 1H, J = 9.5 Hz), 7.15 (d, 1H), 7.39 (s, 1H), 7.64 (d, 1H, J = 7.7 Hz), 7.77 (m, 2H), 7.88 (d, 1H, J = 9.5 Hz), 9.1 (s, 1H), 10.9 (s, 1H) ; MS (ESI) : 503 (M + H⁺) and 525 (M + Na⁺).

20 Example 14

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(quinoxalin-5'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide



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The general procedure of Example 13, using 5-Bromomethylquinoxaline^{Ref 7} as the alkylating agent, was followed to give the title compound as a white solid. m.p. = 170-174°C ;
¹H-NMR (DMSO d₆) : 0.75 (s, 9H), 0.79 (d, 3H, J = 6.2 Hz), 0.85 (d, 3H, J = 6.2 Hz), 0.93 (m, 1H), 1.35-1.5 (m, 2H), 2.53 (d, 3H, J = 4.4 Hz), 3.03 (m, 1H), 3.94 (d, 1H, J = 9.9 Hz), 4.14 (d, 5 1H, J = 9.5 Hz), 4.96 (d, 1H), 5.1 (d, 1H), 7.75-7.81 (m, 3H), 7.9 (d, 1H, J = 7.3 Hz), 8.01 (d, 1H, J = 8.4 Hz), 8.92 (d, 1H, J = 1.8 Hz), 8.97 (d, 1H, J = 1.8 Hz), 9.12 (s, 1H), 10.98 (s, 1H) ;
MS (ESI) : 474 (M + H⁺) and 496 (M + Na⁺).

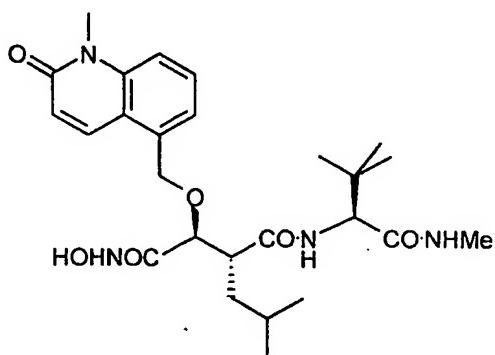
Purification : C18 preparative HPLC using as eluant a mixture of acetonitrile and water / 1 % AcOH (gradient from 1/5 to 1/1) to give the title compound (82 mg, 43 %).

10

Example 15

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-5'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.

15



The general procedure of Example 13, using 5-Bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline^{Ref 8} as the alkylating agent was followed to give the title compound as a white solid. m.p. = 198-200°C ;

20 ¹H-NMR (DMSO d₆) : 0.61 (s, 9H), 0.77 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 0.9 (m, 1H), 1.3-1.5 (m, 2H), 2.52 (d, 3H, J = 4.7 Hz), 2.95 (m, 1H), 3.62 (s, 3H), 3.84 (d, 1H, J = 9.5 Hz), 4.60 (d, 1H, J = 9.2 Hz), 4.45 (d, 1H), 4.7 (d, 1H), 6.62 (d, 1H, J = 9.9 Hz), 7.18 (d, 1H, J = 5.9 Hz), 7.51-7.6 (m, 3H), 7.64 (m, 1H), 8.0 (d, 1H, J = 9.5 Hz), 9.1 (s br, 1H), 10.95 (s, 1H) ; MS (ESI) : 503 (M + H⁺) and 525 (M + Na⁺);

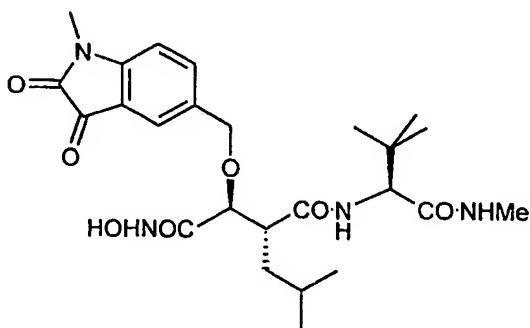
- 43 -

Purification : filtration through C18 'bond-elute' column using as eluant a mixture of methanol and water / 1 % AcOH (3/2) to give the title compound (120 mg, 57 %);

Example 16

5

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2',3'-dioxoindolin-5'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.



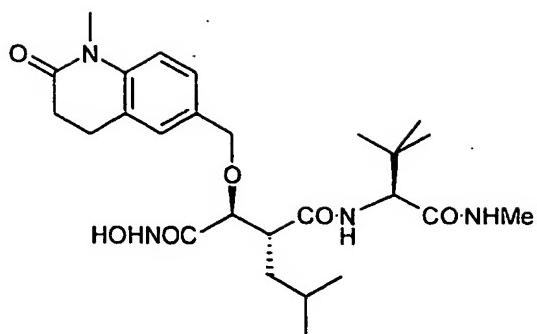
- The general procedure of Example 13, using 5-Bromomethyl-1-methyl-2,3-dioxoindoline^{Ref 9} as the alkylating agent was followed to give the title compound as an orange solid. m.p. = 142-145°C;
- ¹H-NMR (DMSO d₆) : 0.77 (d, 3H, J = 6.6 Hz), 0.81 (s, 9H), 0.82 (d, 3H, J = 6.6 Hz), 0.9 (m, 1H), 1.3-1.45 (m, 2H), 2.54 (d, 3H, J = 4.4 Hz), 2.92 (m, 1H), 3.14 (s, 3H), 3.75 (d, 1H, J = 9.2 Hz), 4.15 (d, 1H, J = 9.5 Hz), 4.23 (d, 1H), 4.38 (d, 1H), 7.07 (d, 1H, J = 8.1 Hz), 7.44 (s, 1H), 7.53 (d, 1H, J = 8.1 Hz), 7.8-7.9 (m, 2H), 9.1 (s br, 1H), 10.9 (s br, 1H) ;
- MS (ESI) : 505 (M + H⁺) and 527 (M + Na⁺).

Purification : C18 preparative HPLC using as eluant a mixture of acetonitrile and water / 1 % AcOH (gradient from 1/9 to 3/7) to give the title compound (10 mg, 34 %).

20 **Example 17**

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'oxo-1',2',3',4'-tetrahydroquinolin-6'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.

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The alkylation step was carried out using 6-bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline, as in Example 1, and the intermediate was reduced with hydrogen over an 5 Adam's catalyst to give the 1,2,3,4-tetrahydroquinoline intermediate which was then deprotected as in Example 13. This provided the title compound as a white solid.

m.p. = 148-150°C ;

¹H-NMR (DMSO d₆) : 0.77 (d, 3H, J = 6.2 Hz), 0.82 (s, 9H), 0.82 (d, 3H), 0.9 (m, 1H), 1.3-1.5 (m, 2H), 2.51 (m, 2H), 2.55 (d, 3H, J = 4.4 Hz), 2.8 (m, 2H), 2.9 (m, 1H), 3.23 (s, 3H), 3.76 (d, 10 1H, J = 9.9 Hz), 4.20 (m, 2H), 4.36 (d, 1H), 6.99 (d, 1H, J = 8.1 Hz), 7.18 (m, 2H), 7.68 (d, 1H, J = 9.5 Hz), 7.76 (m, 1H), 9.1 (s br, 1H), 10.9 (s br, 1H) ;
MS (ESI) : 505 (M + H⁺) and 527 (M + Na⁺).

Purification : C18 preparative HPLC using as eluant a mixture of methanol and water / 1 % AcOH (gradient from 3/7 to 7/3) to give the title compound (146 mg, 53 %).

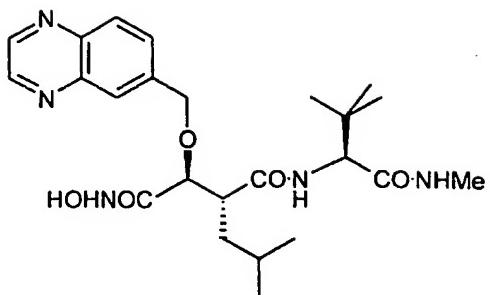
15

Example 18

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(quinoxalin-6'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.

20

- 45 -



The general procedure of Example 13, using 6-Bromomethylquinoxaline^{Ref 10} as the alkylating agent was followed to give the title compound as a white solid.

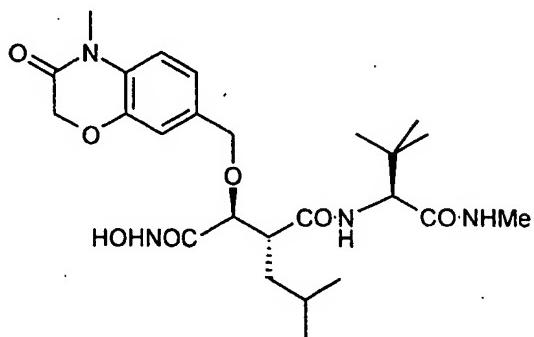
m.p. = 156-160°C ;

5 ¹H-NMR (DMSO d₆) : 0.77 (s, 9H), 0.78 (d, 3H), 0.84 (d, 3H, J = 6.2 Hz), 0.9 (m, 1H), 1.38-1.5 (m, 2H), 2.53 (d, 3H, J = 4.8 Hz), 3.02 (m, 1H), 3.87 (d, 1H, J = 9.9 Hz), 4.19 (d, 1H, J = 9.2 Hz), 4.51 (d, 1H), 4.69 (d, 1H), 7.76 (m, 3H), 8.02 (m, 2H), 8.93 (m, 2H), 9.1 (s br, 1H), 10.9 (s br, 1H) ; MS (ESI) : 474 (M + H⁺) and 496 (M + Na⁺).

Purification : C18 preparative HPLC using as eluant a mixture of acetonitrile and water / 1 %
10 AcOH (gradient from 1/9 to 1/2) to give the title compound (87 mg, 34 %).

Example 19

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,3-dihydro-4-methyl-3-oxo-1,4-benzoxazin-7-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.



The general procedure of Example 13, using 7-Bromomethyl-2,3-dihydro-4-methyl-3-oxo-1,4-benzoxazine^{Ref 11} as the alkylating agent was followed to give the title compound as
20 a white solid. m.p. = 132-138°C ;

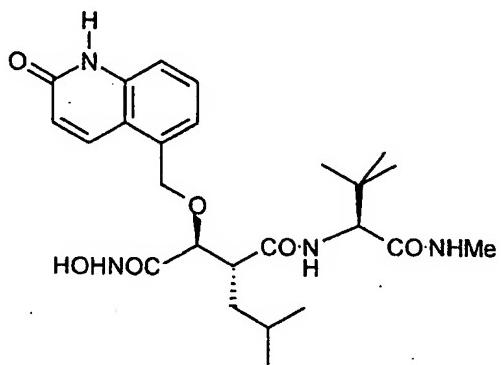
¹H-NMR (DMSO d₆) : 0.77 (d, 3H, J = 6.2 Hz), 0.83 (d, 3H), 0.84 (s, 9H), 0.89 (m, 1H), 1.3-1.45 (m, 2H), 2.54 (d, 3H, J = 4.4 Hz), 2.92 (m, 1H), 3.26 (s, 3H), 3.75 (d, 1H, J = 9.5 Hz), 4.20 (m, 2H), 4.36 (d, 1H), 4.61 (s, 2H), 6.92 (m, 2H), 7.06 (d, 1H, J = 8.1 Hz), 7.69 (d, 1H, J = 9.5 Hz), 7.76 (m, 1H), 9.1 (s br, 1H),
 5 10.83 (s, 1H); MS (ESI) : 507 (M + H⁺) and 529 (M + Na⁺).

Purification : filtration through C18 'bond-elute' column using as eluant a mixture of methanol and water / 1 % AcOH (1/1) to give the title compound (155 mg, 64 %);

Example 20

10

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2'-oxo-1',2'-dihydroquinolin-5'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.



15

The general procedure of Example 13, using 5-Bromomethyl-2-oxo-1,2-dihydroquinoline ^{Ref 12} as alkylating agent [in the presence of 2 equivalents of base] was followed to give the title compound as a white solid.

¹H-NMR (DMSO d₆) : 0.61 (s, 9H), 0.77 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.2 Hz), 0.9 (m, 1H), 1.25-1.45 (m, 2H), 2.52 (d, 3H, J = 4.8 Hz), 2.9 (m, 1H), 3.83 (d, 1H, J = 9.5 Hz), 4.05 (d, 1H, J = 9.2 Hz), 4.4 (d, 1H), 4.66 (d, 1H), 6.5 (d, 1H, J = 9.9 Hz), 7.06 (d, 1H, J = 6.9 Hz),
 20 7.25 (d, 1H, J = 8.4 Hz), 7.34 (t, 1H, J = 7.3 Hz), 7.56 (d, 1H, J = 9.1 Hz), 7.69 (m, 1H), 7.99 (d, 1H, J = 9.9 Hz), 9.1 (s br, 1H), 10.9 (s br, 1H), 11.7 (s, 1H);
 MS (ESI) : 489 (M + H⁺) and 511 (M + Na⁺).

Purification : C18 preparative HPLC using as eluant a mixture of methanol and water / 1 %

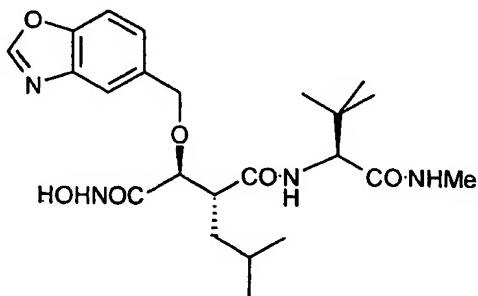
25 AcOH (gradient from 1/4 to 2/1) to give the title compound (112 mg, 37 %).

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Example 21

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzoxazol-5'-yl)methoxysuccinyl]-L-tert-leucine-N¹-methylamide.

5



The general procedure of Example 13, using 5-Bromomethylbenzoxazole^{Ref 13} as the alkylating agent was followed to give the title compound as a white powder.

m.p. = 120-124°C ;

10 ¹H-NMR (DMSO d₆) : 0.77 (d, 3H, J = 6.2 Hz), 0.79 (s, 9H), 0.83 (d, 3H, J = 6.2 Hz), 0.89 (m, 1H), 1.3-1.5 (m, 2H), 2.54 (d, 3H, J = 4.4 Hz), 2.94 (m, 1H), 3.81 (d, 1H, J = 9.9 Hz), 4.17 (d, 1H, J = 9.5 Hz), 4.36 (d, 1H), 4.54 (d, 1H), 7.3 (d, 1H, J = 8.4 Hz), 7.6-7.8 (m, 4H), 8.71 (s, 1H), 9.1 (s br, 1H), 10.9 (s br, 1H) ; MS (ESI) : 463 (M + H⁺) and 485 (M + Na⁺).

Purification : C18 preparative HPLC using as eluant a mixture of acetonitrile and water / 1 %

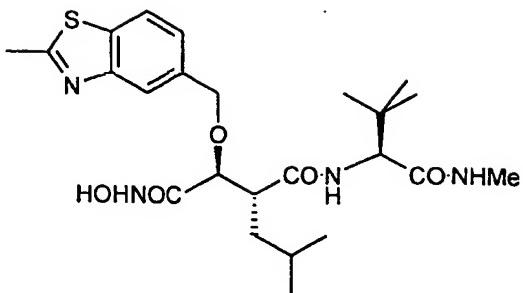
15 AcOH (gradient from 1/9 to 1/3) to give the title compound (22 mg, 17 %).

Example 22

N²-[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2'methylbenzothiazol-5'-yl)methoxysuccinyl]-L-

20 tert-leucine-N¹-methylamide.

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The general procedure of Example 13, using 5-Bromomethyl-2-methylbenzothiazole ^{Ref 14} as the alkylating agent was followed to give the title compound as a white solid.

m.p. = 133-136°C ;

- 5 ¹H-NMR (DMSO d₆) : 0.77 (d, 3H, J = 6.6 Hz), 0.8 (s, 9H), 0.83 (d, 3H, J = 6.6 Hz), 0.88 (m, 1H), 1.35-1.5 (m, 2H), 2.54 (d, 3H, J = 4.4 Hz), 2.79 (s, 3H), 2.95 (m, 1H), 3.8 (d, 1H, J = 9.5 Hz), 4.18 (d, 1H, J = 9.2 Hz), 4.37 (d, 1H), 4.55 (d, 1H), 7.27 (d, 1H, J = 8.4 Hz), 7.65-7.8 (m, 3H), 7.93 (d, 1H, J = 8.1 Hz), 9.1 (s, 1H), 10.86 (s, 1H) ;
MS (ESI) : 493 (M + H⁺) and 515 (M + Na⁺).

- 10 Purification : C18 preparative HPLC using as eluant a mixture of acetonitrile and water / 1 % AcOH (gradient from 1/9 to 1/3) to give the title compound (110 mg, 40 %).

Example 23

- 15 Typical tablet formulations for a compound of this invention or a pharmaceutically-acceptable salt thereof ('Compound X') are:

(a)	<u>Tablet Formulation 1</u>	<u>mg/tablet</u>
	Compound X.....	100
20	Lactose Ph.Eur.....	179
	Croscarmellose sodium.....	12
	Polyvinylpyrrolidone.....	6
	Magnesium stearate.....	3

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(b)	<u>Tablet Formulation II</u>	<u>mg/tablet</u>
	Compound X.....	250
	Lactose Ph.Eur.....	215
	Croscarmellose sodium.....	20
5	Polyvinylpyrrolidone.....	10
	Magnesium stearate.....	5

The tablets may be prepared by conventional procedures well known in the pharmaceutical art and may be film coated with typical coating materials such as hydroxypropylmethylcellulose.

10

References for Starting Materials

Ref 1. : T. G. C. Bird and A. Olivier, Bioorg. Med. Chem. Lett., 6 (5), 515-20, 1996.

15 Ref 2. : M. J. Crimmin, P. R. Beckett and M. H. Davis, Patent WO 94/21625, 1994.

Ref 3. : Prepared by treatment of commercially available 8-bromomethylquinoline with sodium iodide in acetone.

20 Ref 4. : L R. Hughes, A. L. Jackman, J. Oldfield, R. C. Smith, K. D. Burrows, P. R. Marsham, A. M. Bishop, T. R. Jones, B. M. O'Conner and A. H. Calvert, J. Med. Chem., 33 (11), 3060, 1990.

Ref 5. : M. R. Gowravaram, J. S. Johnson, D. Delecki, E. R. Cook, B. E. Tomczuk, A. K.

25 Ghose, A. M. Mathiowitz, J. C. Spurlino, B. Rubin et al., J. Med. Chem., 38(14), 2570-81, 1995.

Ref 6. : 7-Bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline was prepared by bromination of 1,7-dimethyl-2-quinolone [RN 67200-70-8; Imperial Chemical Industries PLC, ICI-Pharma S.

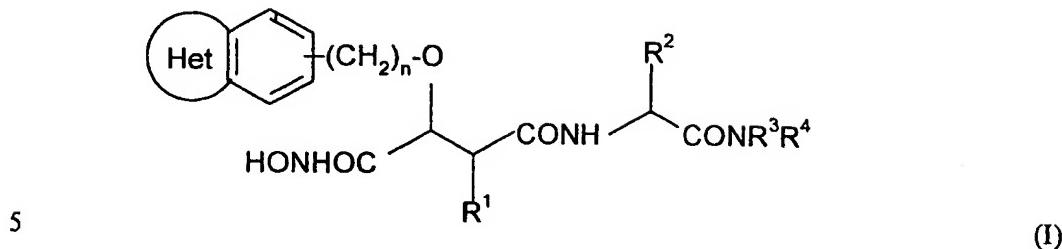
30 A.; (Crawley, G. C., Hamon, A.) Eur. Pat. Appl., EP 385679 A2].

- 50 -

- Ref 7. : 5-Bromomethylquinoxaline - RN 131454-80-3; Kaken Pharmaceutical Co., Ltd.;
(Maeda, T., Take, M., Ishibashi, A., Ariyoshi, T., Yokoo, M.); Jpn. Kokai Tokkyo Koho, JP
02223558 A2.
- 5 Ref 8. : 5-Bromomethyl-1-methyl-2-oxo-1,2-dihydroquinoline was prepared by bromination of
1,5-dimethyl-2-quinolone [RN 67200-69-5; Imperial Chemical Industries PLC, ICI-Pharma S.
A.; (Crawley, G. C., Hamon, A.) Eur. Pat. Appl., EP 385679 A2].
- Ref 9. : 5-Bromomethyl-1-methyl-2,3-dioxoindoline - RN 139487-13-1; Imperial Chemical
10 Industries PLC, ICI-Pharma S. A.; (Bruneau, P. A. R., Crawley, G. C., Oldham, K.) Eur. Pat.
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- Ref 10. : 6-Bromomethylquinoxaline - RN 53967-21-8; Kees, K. L., Caggiano, T. J., Steiner,
K. E., Fitzgerald, J. J., Kates, M. J., Christos, T. E., Kulishoff, J. M., Moore, R. D., McCaleb,
15 M. L.; J. Med. Chem., (1995), 38 (4), 617.
- Ref 11. : 7-Bromomethyl-2,3-dihydro-4-methyl-3-oxo-1,4-benzoxazine - RN 139502-99-1;
Imperial Chemical Industries PLC, ICI-Pharma S. A.; (Bruneau, P. A. R., Crawley, G. C.) Eur.
Pat. Appl., EP 462813 A2.
- 20 Ref 12. : 5-Bromomethyl-2-oxo-1,2-dihydroquinoline - RN 103702-28-9; Uchida, M., Tabusa,
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3775.
- 25 Ref 13. : 5-Bromomethylbenzoxazole - RN 181038-98-2; Rhone Poulenc Rorer Ltd.; (Porter,
B., Smith, C., Walsh, R. J. A., Majid, T. N., McCarthy, C., Harris, N. V., Astles, P. C.,
McLay, I. M., Morley, A. D., et al.) PCT Int. Appl., WO 9622978 A1.
- Ref 14. : 5-Bromomethyl-2-methylbenzothiazole - RN 125872-96-0; Toyama Chemical Co.,
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CLAIMS

1. A compound of the formula (I):



wherein:

n is 1 to 6;

Het is a nitrogen containing ring fused to the benzene ring on two adjacent carbon atoms to form a bicyclic ring system which ring system may be optionally substituted;

10 R¹ is hydrogen, C₁₋₈alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl, heterocyclylC₁₋₆alkyl or C₃₋₈cycloalkylC₁₋₆alkyl;

R² is C₁₋₆alkyl, C₂₋₆alkenyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl or the side-chain of a naturally occurring amino acid;

15 R³ is hydrogen, C₁₋₆alkyl, C₃₋₈cycloalkyl, C₄₋₈cycloalkenyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl or heterocyclylC₁₋₆alkyl;

R⁴ is hydrogen or C₁₋₆alkyl; or R³ and R⁴ together with the nitrogen atom to which they are joined form a heterocyclic ring;

wherein any group or ring, in R¹-R⁴, is optionally substituted;

20 or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof.

2. A compound according to claim 1 wherein Het is a 5- or 6-membered ring containing one or two ring nitrogen atoms.

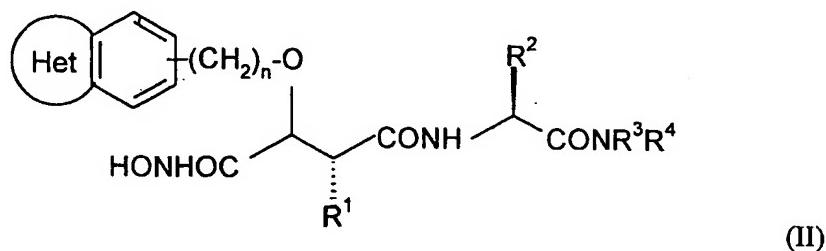
25 3. A compound according to claim 1 wherein Het and the benzene ring to which it is fused forms a quinoline, isoquinoline, quinazoline, 1-methyl-2-oxo-1,2-dihydroquinoline, 2-

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methyl-4-hydroxyquinazoline or 2-methyl-4-hydroxy-7-bromoquinazoline bicyclic ring system.

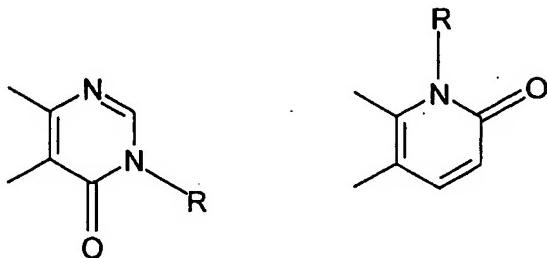
4. A compound according to claim 1 wherein Het and the benzene ring to which it is fused forms a benzoxazole or 2-methylbenzothiazole bicyclic ring system.

5. A compound of the formula (II):



10

wherein n is 1; Het is of the sub-formula (ii) or (iii):



15

(ii)

(iii)

wherein either of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro, bromo or fluoro, C₁₋₆alkyl for example methyl, isopropyl or tert-butyl, C₁₋₆alkoxy for example methoxy, hydroxy, amino, C₁₋₆alkylamino for example methylamino or di-C₁₋₆alkylamino for example dimethylamino; R¹ is isobutyl; R² is isobutyl, tert-butyl or benzyl; R³ is methyl, ethyl, n-propyl, isobutyl, tert-butyl, 2-dimethylaminoethyl or benzyl; and R⁴ is hydrogen or methyl.

6. A compound according to claim 1 which is:

- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- 5 N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- 10 N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(7'-bromo-2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-(2-dimethylaminoethyl)amide;
- 15 N²-[4-(N-hydroxyamino)-2R-(4'-benzyloxy)butyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-dimethylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-
20 (2-dimethylaminoethyl)amide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-(4'-benzyloxy)butyl-3S-(quinolin-8'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- 25 N²-[4-(N-hydroxyamino)-2R-(3'-benzyloxy)propyl-3S-(2'-methyl-4'-oxo-3',4'-dihydroquinazolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-7'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(quinoxalin-5'-yl)methoxysuccinyl]-L-*tert*-leucine-
- 30 N¹-methylamide;

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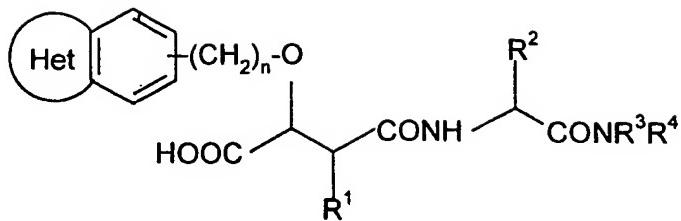
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'-oxo-1',2'-dihydroquinolin-5'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2',3'-dioxoindolin-5'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- 5 N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(1'-methyl-2'oxo-1',2',3',4'-tetrahydroquinolin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(quinoxalin-6'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(2,3-dihydro-4-methyl-3-oxo-1,4-benzoxazin-7'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- 10 N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(2'-oxo-1',2'-dihydroquinolin-5'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(benzoxazol-5'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- 15 N²-[4-(N-hydroxyamino)-2R-isobutyl-3S-(2'methylbenzothiazol-5'-yl)methoxysuccinyl]-L-*tert*-leucine-N¹-methylamide;
- or a pharmaceutically acceptable salt.

7. A pharmaceutical composition which comprises a compound according to any one of
20 claims 1 to 6 and a pharmaceutically acceptable carrier.

8. The use of a compound according to any one of claims 1 to 6 for the manufacture of
a medicament for treating disease conditions mediated by TNF.

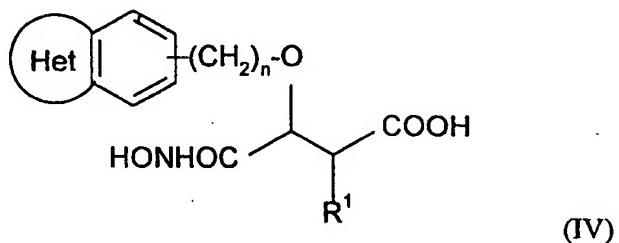
25 9. A process for preparing a compound according to any one of claims 1 to 6 or a
pharmaceutically acceptable salt or in vivo hydrolysable ester thereof which process comprises
a) reacting a compound of the formula (III):

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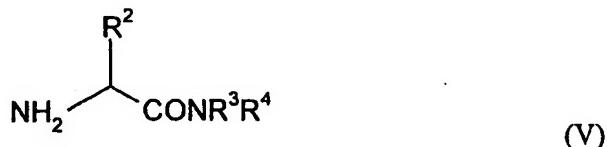
(III)

- wherein n, Het and R¹-R⁴ are as defined in claim 1, or an activated derivative thereof with
 5 hydroxylamine, O-protected hydroxylamine or a salt thereof; or
 b) coupling a compound of the formula (IV) with a compound of the formula (V);



(IV)

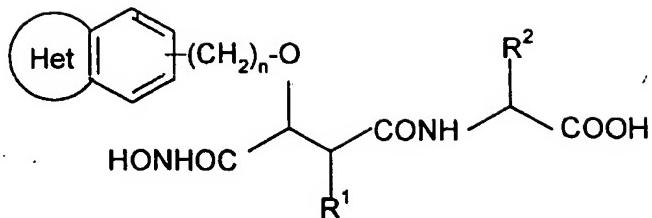
10



(V)

- wherein n, Het and R¹-R⁴ are as defined in claim 1, under standard peptide coupling conditions; or
 15 c) reacting a compound of the formula (VI) with compound of the formula (VII);

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(VI)

5



(VII)

wherein n, Het and R-R⁴ are as defined in claim 1:

wherein any functional group is protected, if necessary, and:

- 10 i. removing any protecting groups;
ii. optionally forming a pharmaceutically acceptable salt or in vivo hydrolysable ester.
10. A compound of the formula (III) as defined in claim 9.

15

20

INTERNATIONAL SEARCH REPORT

Inte
ional Application No
PCT/GB 98/00910

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 6	C07D215/22	C07D215/14	C07D239/90	C07D241/42	C07D209/38
	C07D265/36	C07D263/56	C07D277/64	A61K31/47	A61K31/505
	A61K31/40	A61K31/535	A61K31/42	A61K31/425	

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 10990 A (BRITISH BIOTECHNOLOGY LTD.) 26 May 1994 see page 22, compound no.59, claims ----	1-10
Y	WO 96 25156 A (BRITISH BIOTECH PHARMACEUTICALS LTD.) 22 August 1996 see claims; examples 2,3 ----	1-10
Y	GB 2 268 933 A (BRITISH BIOTECHNOLOGY) 26 January 1994 see claims; examples ----	1-10
Y	WO 95 19961 A (BRITISH BIOTECH PHARMACEUTICALS LTD.) 27 July 1995 see claims; examples -----	1-10

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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"S" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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9 July 1998

17/07/1998

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No

PCT/GB 98/00910

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No

PCT/GB 98/00910

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